STUDIES ON THE COST EFFECTIVE PROCESS FOR CEFIXIME AND CHARECTERIZATION OF RELATED IMPURITIES

THESIS

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JHANSI

BY

DNYANDEV. R. RANE

IN FULFILLMENT OF THE REQUIRMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ORGANIC CHEMISTRY

MARCH 2005

Certificate

This to certify that the entitled **"STUDIES ON THE COST EFFECTIVE PROCESS FOR CEFIXIME AND CHARECTERIZATION OF RELATED IMPURITIES"** submitted in fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY (SYNTHETIC ORGANIC CHEMISTRY)** is a record of bonafied research carried out by DNYANDEV RANE at Lupin Research Park, Pune, under our supervision and the manuscript is suitable for submission for the award of degree of **DOCTOR OF PHILOSOPHY IN ORGANIC CHEMISTRY.**

This to further certify that **DNYANDEV RANE** has put in minimum 200days attendance in the Department of Organic Chemistry during the course of this study.

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Finally, acknowledging my indebtedness to my parent's shri Ragho Rane and my family members for their constant encouragement and moral support.

PUNE

March 2005

(DNYANDEO RAGHO RANE)

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GENERAL REMARKS

1) All temperatures are in °C and are uncorrected.

2) ^{1H} NMR spectra were recorded on DRX-200 spectrometer in $CDCl_3$, DMSO-d_6, D_2O, MeOD, containing TMS as an internal standard with chemical shift (δ) expressed in ppm downfield from TMS. The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and b = broad.

3) Infrared spectra (v max cm^{-1}) were recorded on IR Prestige-21 Shimadzu or Perkin –Elmer, FTIR 1600 series with potassium bromide optics.

4) Mass spectra were recorded at ionization energy of 60-200 e V on LC-MS instrument (PE-Sciex,API-3000).

5) All solvents and reagents were purified and dried by standard procedures. All evaporations were carried out under reduced pressure on Buchi rotary evaporator.

6) TLC was carried out on fluorescent silica gel plates. The plates were analyzed under ultra violate light chamber.

7) Column chromatography was performed on resin XAD-118O in aqueous media.

8) The compound numbers, scheme numbers and reference numbers given in each chapter refer to that particular chapter only.

ABBREVIATIONS

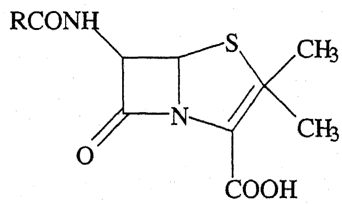
MeCHO	Acetaldehyde
AcOH	Acetic acid
$AlCl_3$	Aluminum chloride
7-ACA	7-Aminocephalosporinic acid
7-AVCA	7-Amino –3-vinyl cephalosporinic acid
7-ADCA	7-Aminodesacetylcephalosporinic acid
7-APCA	7-Amino-3-propenyl cephalosporinic acid
7-APC	7-amino –3-[(1-methyl-1-pyrrolidino)-methyl] ceph-3-em – -4- carboxylate.
NH_3	Ammonia
PhCHO	Benzaldehyde
BSA	N, O-bis-trimethylsilyl acetamide
$CHCl_3$	Chloroform
TMCS	Chlorotrimethylsilane
DMW	De-mineralized water
DATMA	Diethylthiophosphoryl –2-amino-4-thiazolyl-2- [(Z)-t-butoxy-carbonyl] methoxyiminoacetate
DMAc	Dimethyl acetamide
DCM or CH_2Cl_2	Dichloromethane
EtOAc	Ethyl acetate
EDTA	Ethylene diamine tetra acetic acid
HCOH	Formaldehyde

HMDS	Hexamethyldisilazane
HCl	Hydrochloric acid
HPLC	High Pressure Liquid Chromatography
HBr	Hydrogen bromide
PCl_5	Phosphorous pentachloride
TMSI	Iodo trimethyl silane
IPA	Isopropyl alcohol
LiBr	Lithium bromide
MeOH	Methanol
NMP	N-Methylpyrrolidine
DCC	N,N-dicyclohexylcarbodiimide
DMA	N, N-Dimethyl aniline
NMM	N-methylmorpholine
GCLE	7-Phenylacetamido-3-chloromethyl-3-cephem-4-carboxylate p-methoxybezyl ester
GCLH	7-Phenylacetamido-3-chloromethyl-3-cephem-4-carboxylate diphenyl methane ester
3-VBA	4-p-methoxybezyl-3-vinyl-7-phenyl-acetamidocephem carboxylate
H_3PO_4	Phosphoric acid
$COCl_2$	Phosgene
KI	Potassium iodide
K_2CO_3	Potassium carbonate

NaI	Sodium iodide
NaOH	Sodium hydroxide
Na_2CO_3	Sodium carbonate
NaBr	Sodium bromide
H_2SO_4	Sulphuric acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
7-AE Salt	p-Toluenesulphonate salt of p-methoxybezyl-7-amino--3-vinylcephem –4-carboxylate
p-TSA	p-Toluenesulphonic acid
TEA	Triethyl amine
TPP or PPh_3	Triphenyl phoshine
TFA	Trifluoroacetic acid

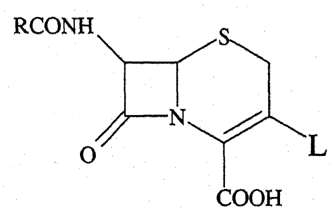
INTRODUCTION

It is over seventy years since Alexander Fleming observed antibiotics[1] between a Penicillium mould and bacterial cultures and gave the name penicillin to the active principle. Although it was proposed in 1943 that penicillin (1) contained a β –lactum ring, this was not generally accepted until an X-ray crystallographic determination of the structure had been completed.



(1)

Penicillin was the first naturally occurring antibiotic to be characterized and used in clinical medicine . It is now seen as the progenitor of the β-lactam family of antibiotics, which are characterized by the possession of the four membered β-lactam ring. Penicillins and Cephalosporin (2) the second member of β-lactam antibiotics family were both originally discovered in fungi but later detected in streptomycetes.

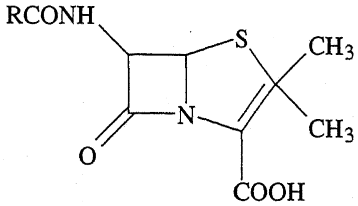


(2)

For R and L see table-1

Until 1970 penicillins and Cephalosporins were the only examples of naturally occurring β-lactum antibiotics. The discovery of 7 α-methoxy cephalosporins (3) from Streptomyces in 1971 stimulated the search for novel β-lactum antibiotics from microbes, both by using sensitive new screening procedures, and by laboratory synthesis. At present, β-lactam antibiotics can be classified in several groups according to their structure:

- Penicillins (penams)(1)
- Cephalosporins (Cephem) (2)
- Cephamycins (3)
- Oxacephems (4)
- Penems (5)
- Oxapepenams such as Clavulanic acid (6)
- Carbapenems such as thienamycin (7)
- Nocardicins (8)
- Monobactams (9)
- Lactivicin (10)



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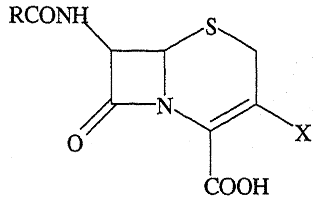

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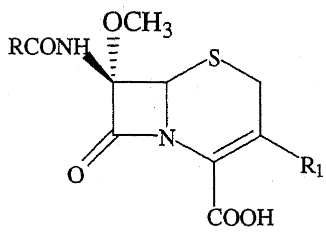

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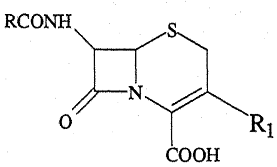

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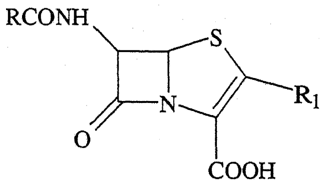

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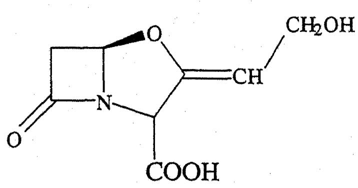

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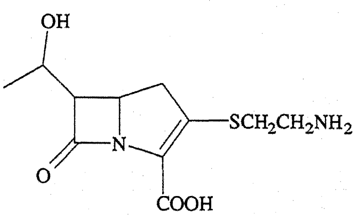

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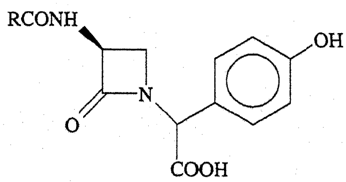

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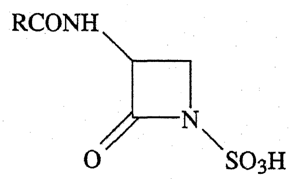

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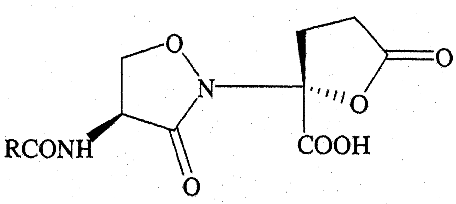
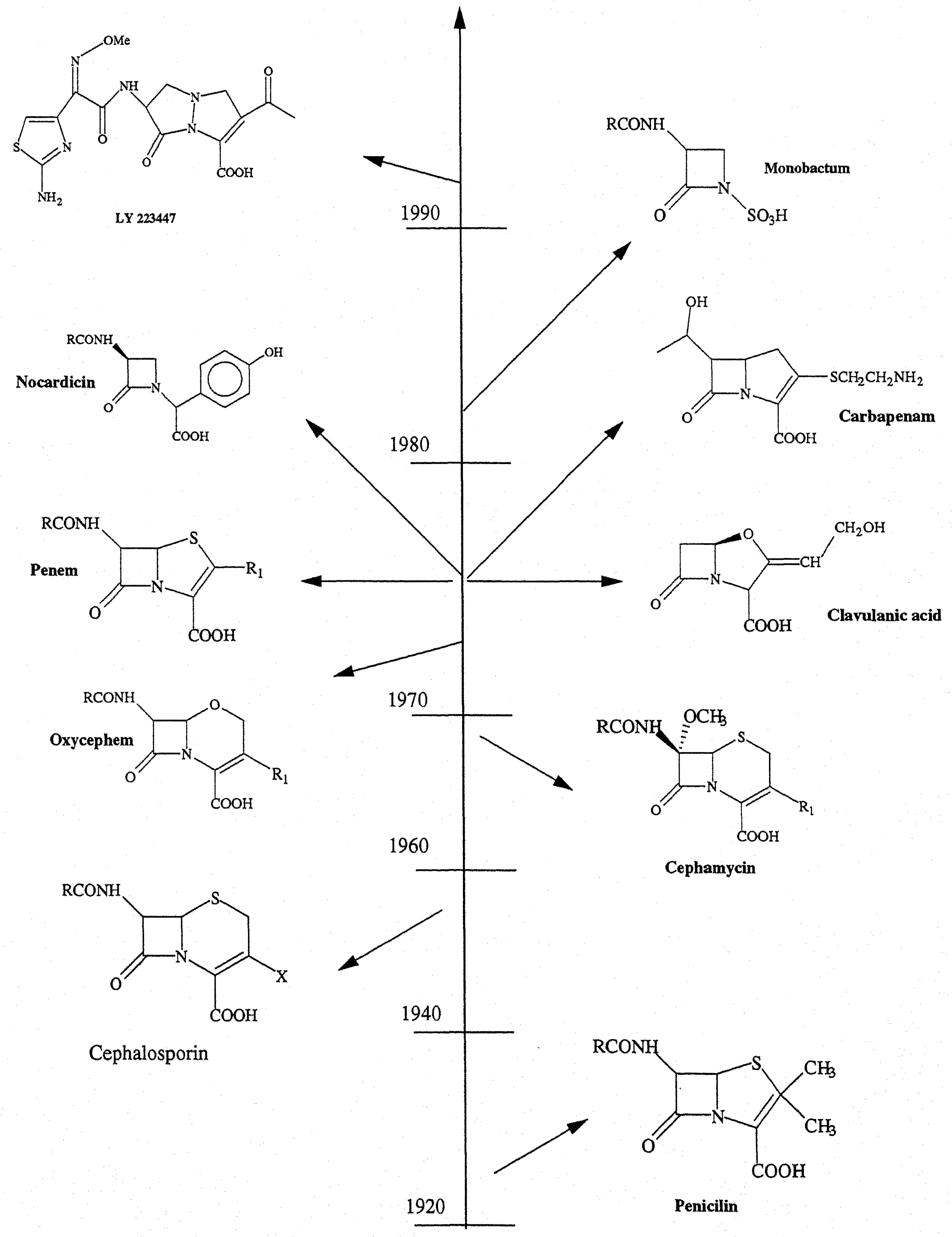


(10)

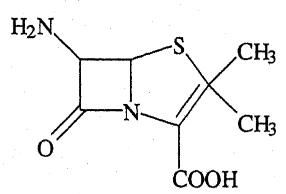
The chronology of these antibiotics is summarized in Figure 1 as shown in below:-

Figure-1



β-Lactam have now been found in eukaryotic fungi, actinomycetes and even in bacteria γ-lactam such as lactivicin (10) and β-lactones, which show antibacterial activity, have also been isolated from various microorganisms.

A large number of nuclear analogues of the β-lactam antibiotics have been prepared by complete chemical synthesis, or by partial synthesis starting from a naturally occurring β-lactam. Most semi synthetic penicillins are made by the acylation of 6-β-aminopenicillinic acid (11), whilst cephalosporins can be prepared to give variety of side chain substituents at C-7 and C-3 (12) classically. β-lactam antibiotics have been named using the ring sulphur as position –1 hence, for cephalosporins and penicillins the numbering is as shown in (2) and (1) respectively. Similarly substitutents in these bycyclic system are usually described by the prefix α- or β- rather than the equivalent exo and endo description. The normal configuration at the three asymmetric centers in penicillin is therefore 3S, 5R and 6R.



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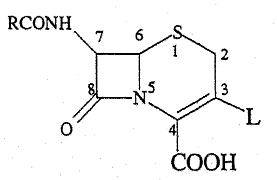

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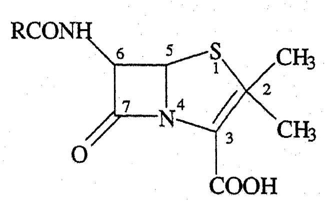
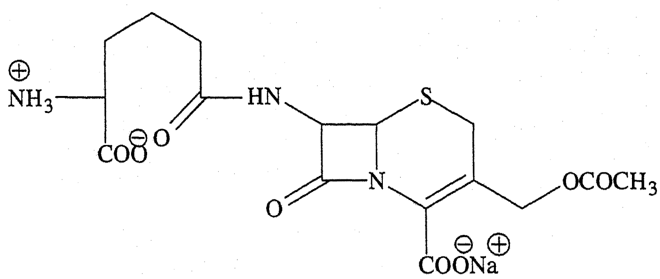


(1)

Occurance of Cephalosporins[2,3,4,5] :-

Cephalosporin C is the parent substance from which the first Cephalosporins to find clinical use were derived. Therefore the requirement of the Cephalosporin C in the industry is increased.



Cephalosporin C

Cephalosporin C is produced in very small amount by wild strain of a species of Cephalosporium [Commonwealth Mycological Institutes Kew (C.M.I) no. 49137], similar to Cephalosporium acrymonium, which was isolated by Brotzu in 1948. Mutant of this strain have been obtained which produced Cephalosporin C in much higher yield.

In addition to Cephalosporin C, the cephalosporium species C.M .I 49137 produced Cephalosporin N, which is related chemically to Cephalosporin C, and an entirely different antibiotic, Cephalosporin P. Cephalosporin N, now known as penicillin N, is identical with an antibiotic, which was formerly named **synnematin B**. Work that began with the study of the latter product has shown that the ability to produce penicillin N is shared by a number of different fungi.

Isolation of Cephalosporin C [6,7] :-

Newton and Abraham et al.[6] first encountered the Cephalosporin C during the chemical study of penicillin N was conducted in September 1953. The penicillin N in a sample of partially purified material was converted to its penillic acid in aqueous solution at pH 2.7 and the resulting product chromatographed on a column of Amberlite IR-4B in ammonium acetate buffer. Cephalosporin C was eluted from the column after the penillic acid and it was readily obtained as a crystalline sodium salt.

The prospect of obtaining the Cephalosporin C in relatively large amount were much improved when the search for higher yielding mutant strain of the cephalosporium species under taken in 1957 by Kelly and his colleague[7] at antibiotic research station Clevedon, began to be rewarding. Mutant 8650, which produced much more Cephalosporin C than wild strain, was used in subsequent fermentation.

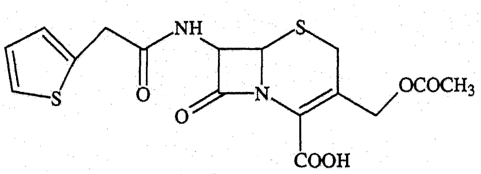
Uses of the Cephalosporin C [8,9]:

Pharmaceutical companies were now showing interest in the Cephalosporins general option for a license had been obtained from N.R.D.C. by E.R.Squibb and company in 1959. In addition to Eli Lilly, three U.S. Companies Merck and Company. Chas. Pfizer and Company, and Smith Kline and French, entered in to option agreements in the following year, as did CIBA in Switzerland and Farmitalia in Italy. In 1961 a similar agreement was made with the Fujisawa Pharmaceutical company in Japan. Until this time it had appeared possible that Cephalosporin C it self might find some clinical use for treatment of penicillin resistant staphylococcal

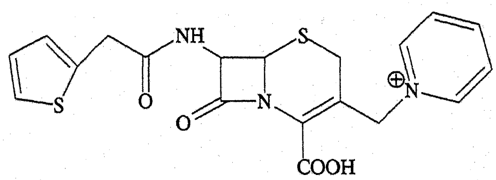
infections, even though its very low specific activity would presumably have required it to be given by intravenous infusion. But this appeared unlikely after the production of 2,6-dimethyoxyphenylpenicillin from 6-aminopenicillanic acid and demonstration of its chemotherapeutic properties (Rolinson et al.[8], 1960;Douthwaite and Trafford,[9] 1960). Thus a great deal depended on the discovery of the method for the 7-aminocephalosporinic acid on large scale.

Side chain cleavage of Cephalosporin C [10,11,12,13] :

Extensive searches in several laboratories for an enzyme, which would remove the D-α–aminoadipyl side chain from Cephalosporin C had no significant success. But before the end of 1960 an ingenious chemical procedure had been discovered in the Eli Lilly Research laboratory which enable the side chain to be removed and 7-aminocephalosporinic acid to be obtained in very much higher yield than was possible by simple hydrolysis (Morin et al.,[10] 1962). By this time Eli Lilly and Glaxo had opened the way to the production of Cephalosporin C in large amount by fermentation. Thus 7-aminocephalosporinic acid became available in quantity and an intensive study of the properties of the derivatives of the compound soon led to introduction of two cephalosporins, namely Cephalothin and Cephaloridine, in to medicine.



Cephalothin



Cephaloridine

The 7-aminocephalosporinic acid was prepared from the Cephalosporin C by side chain cleavage. And it was cleaved by two methods namely by enzymatic (Walton 1964 [12]) and other by chemically (Loder et al 1961).

Subsequent patents issued to Nethelandsche Gistern Spiritus Fabriek (Belg. Patent 718,824), Glaxo Labs (Belg. Patent 719,712) and CIBA limited (Neth Patent 68,12413) described a successful phosphorous pentachloride cleavage of Cephalosporin C using silylating agents to simultaneously protect the amino carboxylic groups.

The side chain elimination of the Cephalosporin C was best achieved in methylene chloride or chloroform solvents in which these fully protected derivatives of Cephalosporin C are mostly soluble The reaction scheme for converting these derivatives to 7-ACA is represented in a general way in the equation below.

Phosphorous pentachloride was recognized to be a more effective chlorinated agent. . In the presence of excess pyridine phosphorous pentachloride gave nearly complete conversion 7-amide function to imino chloride in few hours at room temperature or even in the cold, and afforded higher yields of 7-ACA esters. On treatment with an alcohol, the imino chloride was converted imino ether, which hydrolyzed with the ease of to an ester of 7-ACA and presumably an N-acylaminoadipic acid diester. A separate and more rigorous hydrolysis of the ester-protecting group, afforded 7-ACA. The 7-ACA was isolated as a crystalline precipitate from aqueous solution at a pH near 3.5, its isoelectric point.

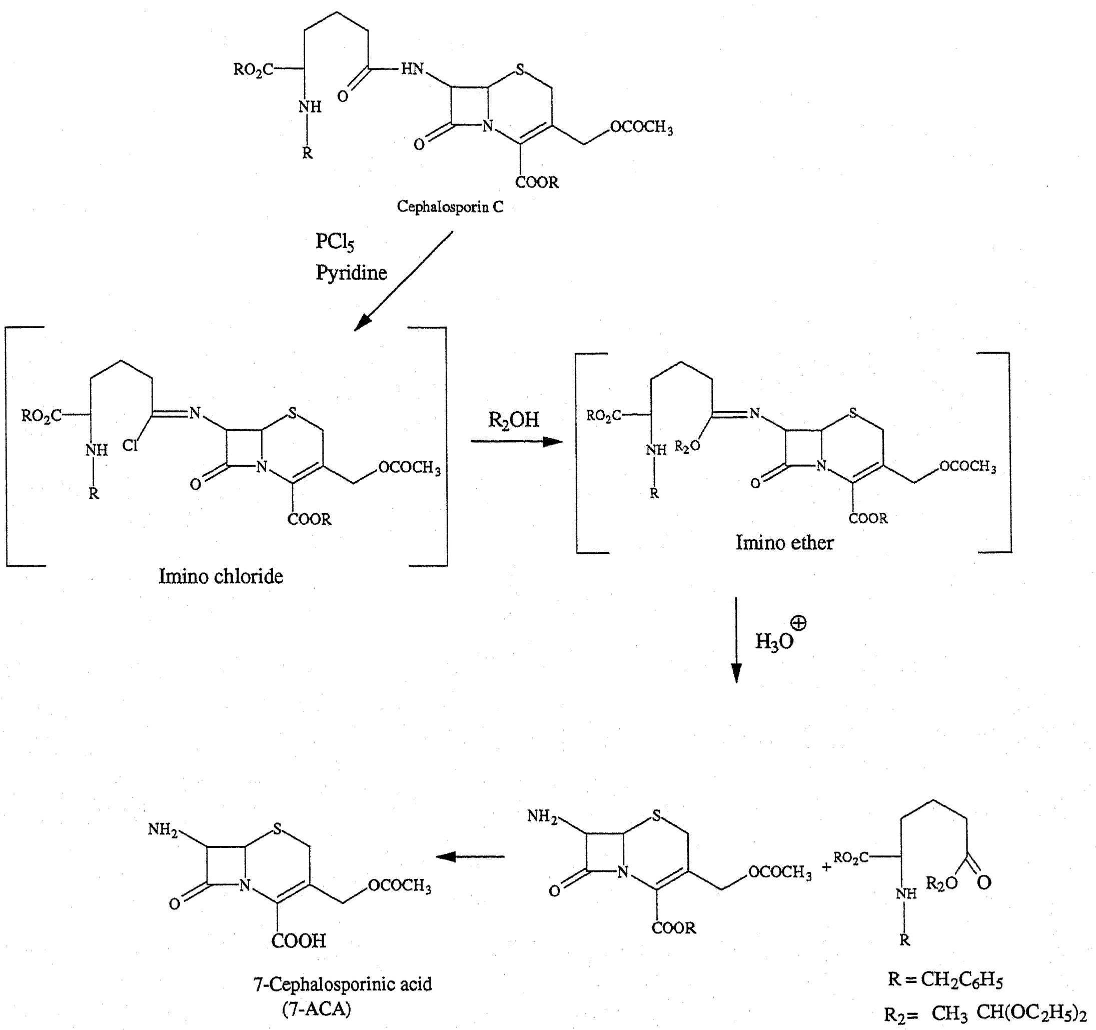


Figure 2

Modification at C-7 amino position affect antibacterial and β-lactamase activity while substitution at C-3 affecting metabolism and pharmakinatics of the Cephalosporins to greater extent than antibacterial activity and carboxylic group in the cephalosporin improve oral absorption.

Based on the activity of the Cephalosporin molecule they are classified in to four categories namely first, second, third and fourth generation antibiotics.

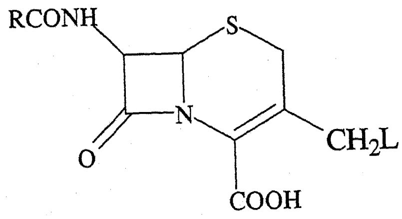
First generation: Active against most gram positive and some gram negative bacteria.

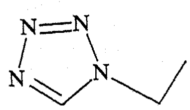
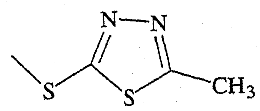
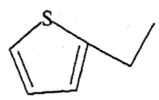
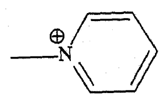
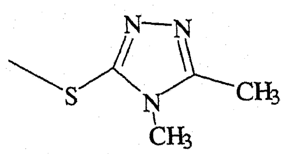
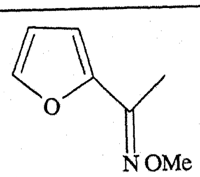
Second generation: Enhanced activity against a greater variety of gram-negative bacteria as compared first generation.

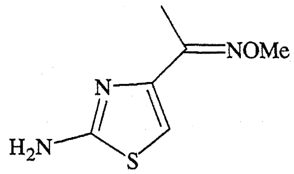
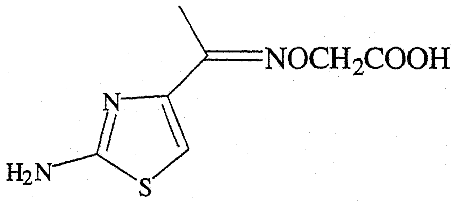
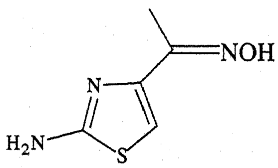
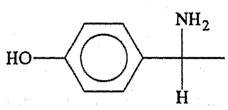
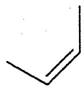
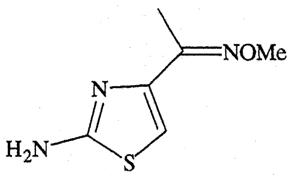
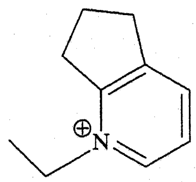
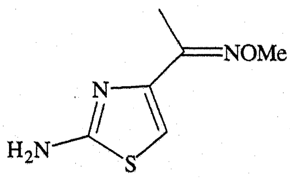
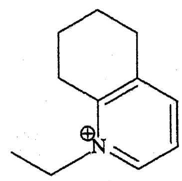
Third generation: These third generation Cephalosporins are distinct from the older β-lactam antibiotics in their intensive antibacterial activity against the wide range of gram-negative bacteria. The exceptional antibacterial activity of the third generation Cephalosporins has been shown to be based on both their enhanced affinity for the target enzymes and their high stability to β lactamase. That Cefixime shares similar characteristic with the other third generation Cephalosporins.Although the Cefixime is less active against staphylococci than are other orally active β-lactum antibiotics, it for more potent against a wide range of gram negative bacteria

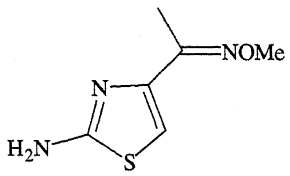
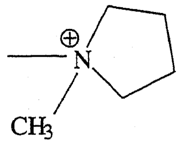
Forth generation: fourth generation injectable Cephalosporin antibiotic with an improved stability to β-lactamases and a broader spectrum of activity than third generation Cephalosporin. Thus a series of α-7 alkoxyimino derivatives having quaternized ammonium group in the 3- side chain was found to be most promising in view of its antimicobial spectrum and other other biological properties.

Some examples of clinically used Cephalosporins are given below in table –1



Cephalosporin	R	L
First generation Cephalosporin		
Cefazolin		
Cephalothin		OAc
Cephaloridine		
Second generation Cephalosporin		
Cefamandole	PhCH(OH)-	
Cefuroxime		—O—CONH$_2$
Cephamycin	HOOC—CH(NH$_2$) (CH$_2$)$_3$—	—O—CONH$_2$

Third generation Cephalosporin		
Cefotaxime		—OAc
Cefixime		$=CH_2$
Cefdinir		$=CH_2$
Cefprozil		
Fourth generation Cephalosporin		
Cefpirome		
Cefquinome		

Cefepime		

Development of the industrial preparation of β-lactum antibiotics was governed by following themes.

- Minimal handling and manipulation of the nucleus because of the sensitive character of the β-lactum moiety and the high prices for these molecules.
- Side chain activation as acid chlorides or mixed anhydride.
- Side chain protection as the Dane salts.
- Active thioester of 2-mercaptobenzothiazole with 2-aminothiozolyl side chain
- Other active ester of 2-aminothiozolyl-side chain.

On the basis of market demand and high profitability of these molecules such as Cefixime, Cefprozil and Cefepime have selected for process development and the work is incorporated in this thesis.

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Objective and strategy

An object of the present work is to provide a convenient and cost effective process for the preparation of the compound Cefixime Trihydrate, Cefprozil monohydrate and Cefepime hydrochloride by reducing number of steps and utilizing cheaper raw materials.

An another objective of the work is to identify ,synthesize and characterize the known and unknown impurities formed during the synthesis of these cephalosporin molecules which are mentioned below.

Cefixime Trihydrate:

The present work also provides a highly selective method for preparation of compound Cefixime Trihydrate) in high yield and high purity, substantially free of impurities, which is simple, convenient and cost-effective and more importantly does not suffer from the limitations associated with the prior art methods.

It is yet another object of the present work to synthesize and characterize the impurities formed during the preparation of Cefixime

Cefprozil monohydrate :

The object of the present work is to synthesize Cefprozil in high purity, substantially free of impurities by a simple and cost-effective industrially feasible method, which comprises preparation of mixed acid anhydride and its condensation with a protected 7-APCA

The present work also provides an improved method of preparation of mixed acid anhydride by selecting the sequence and temperature of addition of the reagents, which will result in minimization of impurities.

To develop cost-effective process for the preparation of Cefprozil in high purity and yield by minimization of the impurities which are associated with the reported methods with concurrent improvement in the purity and yield of the product. The steps involved in the reported synthesis of Cefprozil monohydrate are minimized and improving yield and reduction of reactant used reduced the manufacturing cost.

Cefepime hydrochloride:

Because of its therapeutic usefulness and efficient broad spectrum of activity, there is always a need for improvement in the process which would result in a product with high purity and yield, with minimum level of impurities, preferably absent, coupled with ease of operation and, more importantly, with low production cost.

The objective of the present work is to provide a novel intermediate useful for the preparation of Cefepime and to provide an improved process for the preparation of Cefepime hydrochloride which is operationally simple in good yield and high purity.

Chapter -II

Process for manufacture of Cefixime trihydrate and synthesis of its impurities

2.1- Introduction

2.2 Literature methods for the preparation of Cefixime trihydrate

2.3 Objective

2.4Present work

2.4.1 selection of method

2.4.2 Preparation of Cefixime trihydrate

2.4.3 Synthesis of Cefixime impurities

2.5 Conclusion.

2.6 Experimental

2.7 References

2.1 Introduction

Cefixime is the first member of third generation orally active cephalosporins[1] These third generation Cephalosporins are distinct from the older β-lactam antibiotics in their intensive antibacterial activity against the wide range of gram negative bacteria. The exceptional antibacterial activity of the third generation Cephalosporins has been shown to be based on both their enhanced affinity for the target enzymes and their high stability to β- lactamase. That Cefixime shares similar characteristic with the other third generation Cephalosporins.Although the Cefixime is less active against staphylococci than other orally active β-lactam antibiotics, it is more potent against a wide range of gram-negative bacteria.

The amino thiazole ring appears to be associated with both excellent activity and oral absorption and the amino group in the thiazole ring is essential for the potential antibacterial activity. Cefixime exhibits geometrical isomerism with reference to the configuration of the oxime. The Z-isomer is predominant isomer relative to E-isomer and is the article of the commerce used in the preparation of the dosage form. The E- isomer is considered as concominent component of Cefixime and is therefore not considered as an impurity in the usual pharmacopoeial sense. The antimicobial activity and oral absorbability of both isomer have been studied in detail. The E – isomer is reported to be 2-32 times less active than the Z- isomer against gram – negative bacteria, although both isomers show appreciable oral absorbability regardless of the configuration of the oxime.

In marked contrast to other Cephalosporin antibiotics Cefixime has vinyl group at 3-position and a (Z)-2-(2-amino-4-thiazolyl)-2-(carboxy methoxyimino) acetyl moiety at C-7 which influences its improved activity against gram negative bacteria and pharmacokinetic properties.

Cefixime is a commercially valuable and therapeutically useful oral Cephalosporin antibiotic effective in controlling diseases caused by a wide variety of Gram positive and Gram-negative microorganisms.

Because of its therapeutic usefulness and efficient broad spectrum of activity, there is always a need for an improved synthetic process which would result in a product with high purity and yield, with minimum level of impurities, preferably absent, coupled with ease of operation and, more importantly, with low production cost.

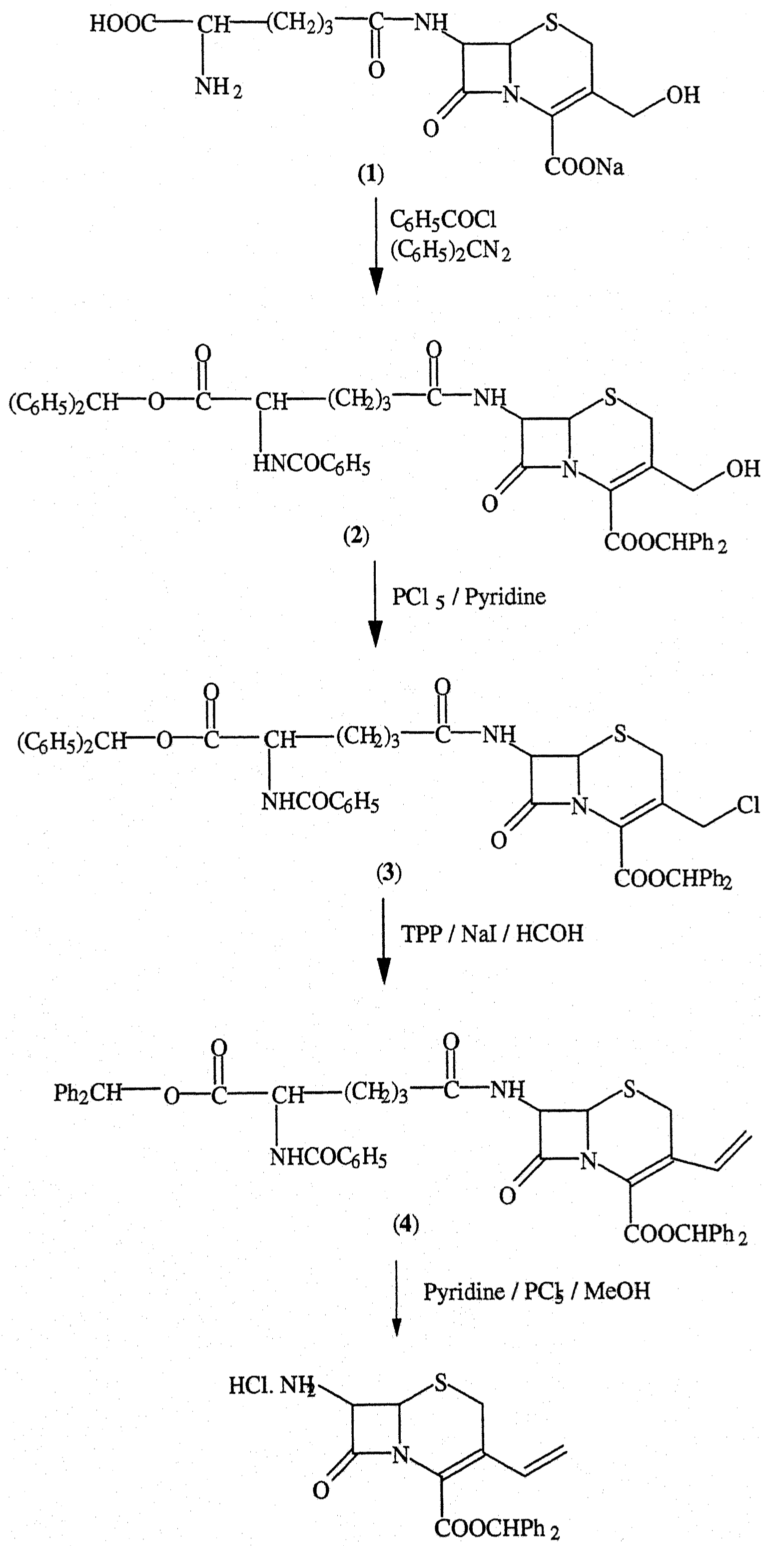
2.2 Literature methods for the preparation of Cefixime trihydrate:

The syntheses of Cefixime reported by Takao Takaya, et al[2] reported the preparation of an advanced key intermediate (**5**), diphenylmethyl-7-amino-3-vinyl-3-cephem-4-carboxylate (7-Amino ester) which is isolated in the form of salts and its converted to Cefixime (**9**). Various synthetic routes for preparation of 7-Amino ester and Cefixime are given as below:

The synthesis (Scheme **–2.1A**) in first part involves the preparation of 7-Amino ester (5) efficiently by treating deacetyl cephalosporin C sodium Salt (**I**) with benzoyl chloride, followed by the reaction with diphenyldiazomethane to give protected deacetylcephalosporin C (2). The reaction of intermediate (**2**) with phosphorous pentachloride and pyridine converts the hydroxymethyl group into a chloromethyl group (**3**). 3-Chloromethyl derivative is then treated with triphenyl phosphine and

sodium iodide in N, N-Dimethylformamide to yield a phosphonium salt, which is then treated with formaldehyde in methylene chloride (by Wittig reaction) to obtain the 3-vinyl Cephem (**4**). Deacylation of Wittig product achieved by treatment with phosphorous pentachloride and pyridine followed by methanol to give the intermediate, 7-Amino ester (**5**).

SCHEME 2.1A



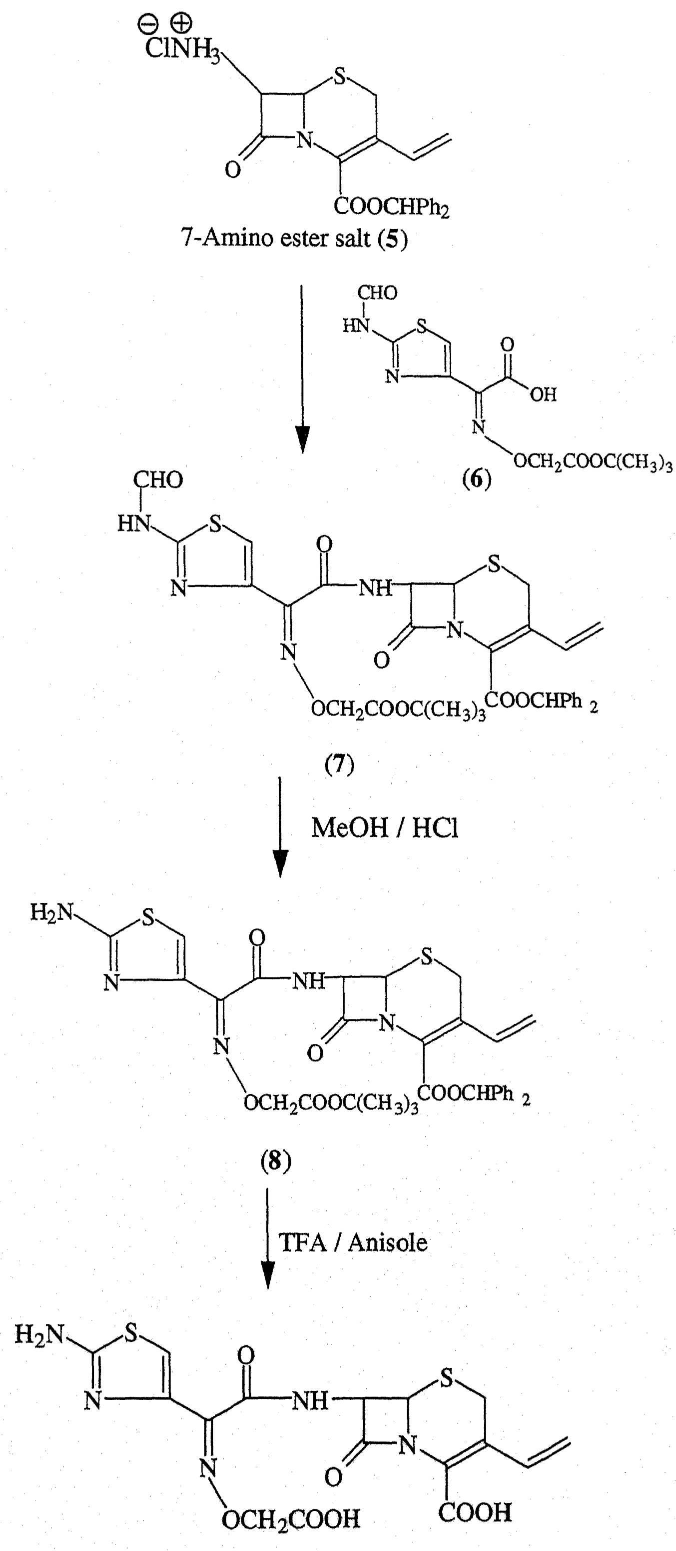
7-AMINO ESTER SALT (5)

In the second part, the above intermediate, 7-Amino ester (**5**) is converted in to Cefixime[3] using two different synthetic routes namely Scheme **2.1B(I**) and Scheme **2.1B(II).**

In scheme **2.1B(I),** the side chain, (Z)–2-(2-Formamido-4-thiazolyl)–2-(*tert*-butoxycorbonylmethoxyimino) acetic acid (**6**) has been activated as its acid chloride derivative by reacting with phosphoryl chloride and Dimethyl formamide and then condendensed with 7-Amino ester (**5**) to give protected Cefixime (**7**). Deprotection of N-formyl group of (**7**) with methanolic hydrochloric acid afforded Cefixime diester (**8**), which was further, converted to Cefixime (**9**) by deesterification with trifluoroacetic acid and anisole.

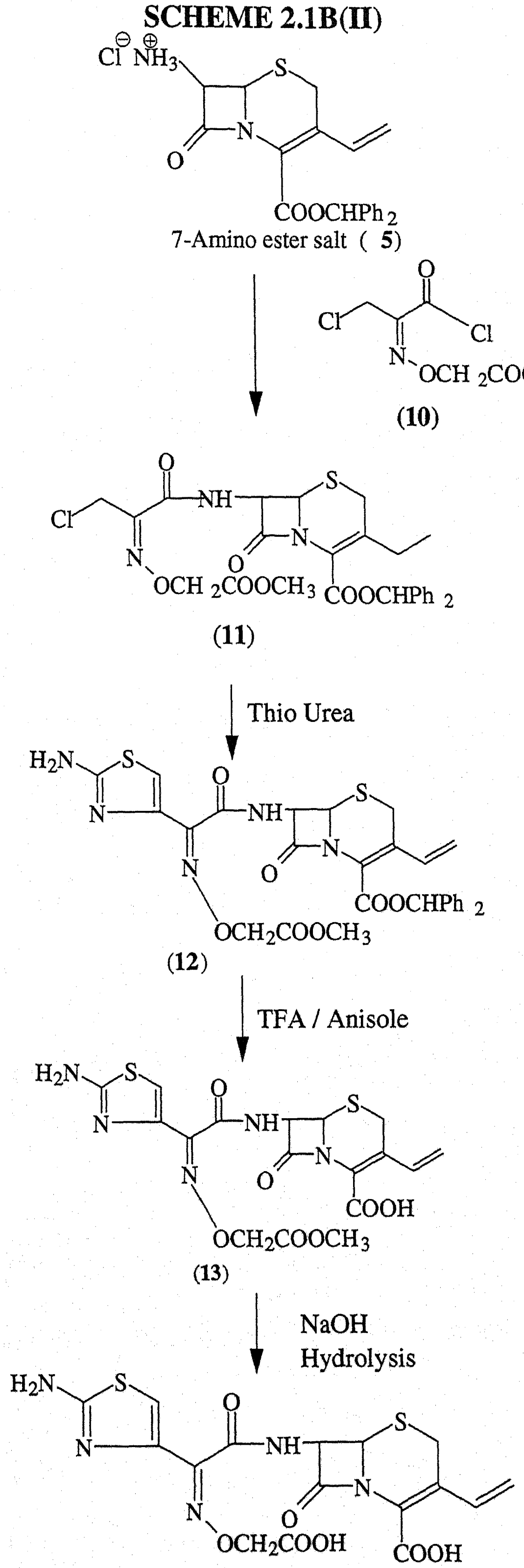
In scheme **2.1B(II),** 7-Amino ester (**5**) was acylated with 4-Chloro-2-methoxycarbonylmethoxyimino-3-oxobutyric acid (**10**) to give acylated Cephem intermediate (**11**). This compound (**11**) was then treated with thiourea to give Intermediate (**12**). The cleavage of diphenylmethyl ester (**12**) of was achieved by treating with trifluoroacetic acid and anisole to give Cefixime monoester (**13**) which on hydrolysis with sodium hydroxide yielded Cefixime (**9**)

SCHEME 2.1B(I)



Cefixime (9)

SCHEME 2.1B(II)



7-Amino ester salt (**5**)

(10)

(11)

(12)

(13)

Cefixime (**9**)

Synthetic Scheme – 2.2

Takao Takaya, et al[4] described the process for the preparation of Cefixime from the starting material, 7-ACA (**14**). In this scheme 7-ACA was deacetylated followed by schiffs base formation with salicyldehyde and esterification with diphenyldiazomethane to give 3-hydroxy derivative (**15**). The above compound (**15**) was converted to (**5**) via (**16**) by the Wittig reaction first making the phosphnium ylide of the 3-hydroxy group followed by converting to an alkene by formaldehyde. The resulting intermediate (**5**) was reacted with (**10**) followed by thiourea to provide the intermediate (**12**) with 7-side chain in place. **12** on hydrolysis resulted in the Cefixime (**9**).

The method of reported by Takao Takaya, et al[4] suffers from a limitation that it utilizes expensive reagent like iodine and the yield is low.

Synthetic Scheme-2.3

Kaplan, et al[5]. described the process for the activation of side chain 2-(2-amino-4-thiazolyl) 2-[(Z)-t-butoxycarbonylmethoxyimino-acetic acid (**17**) by reacting with dibenzothiazol-2-yl disulfide (**18**) in the presence of triphenyl phosphine and triethyl amine which was afforded reactive ester 2-(2-amino-4-thiazolyl) 2-[(Z)-t-butoxycarbonylmethoxyimino-acetic acid 2-benzothiazolyl thioester (**19**).

This activated side chain (**19**) was used for preparation of Cefixime by condensation with the required β-lactam unit but removal of 2-mercaptobenzthiazole, a byproduct liberated from the reaction, was found to be difficult. Removal of the same at higher pH resulted in extensive emulsion formation and lead to the decomposition of the condensed product.

Synthetic Scheme-2.4

Wsung Kyurn Kim, et.al[6]. reported the another process for the activation of side chain 2-(2-amino-4-thiazolyl) 2-[(Z)-t-butoxycarbonylmethoxyimino-acetic acid(**17**) by reacting with diethylthiophosphoryl chloride (**20**) in presence of tri-n-butyl amine which is afforded the reactive thiophosphate derivative of 2-(2-amino-4-thiazolyl)2-[(Z)-t-butoxycarbonylmethoxyimino-acetic acid (**21**) and used in for the preparation of Cefixime.

Yutaka Kameyama et. al[7] described the method of introducing a vinyl group at C_3 position of the cephem compound in a one pot Wittig reaction of 3-chloromethylcephem compound (**22**) with triphenyl phosphine and NaI or KI in a solvent mixture of dichloromethane and acetone or tetrahydrofuran or dioxane; treating the resulting solution with a aqueous solution of 10% sodium bicarbonate, and aqueous solution of formaldehyde, or acetaldehyde or chloroacetaldehyde at a temperature of 22 to 25°C. The yields obtained are in the range of 85-95 %.

The method has the disadvantage that it utilizes alkali metal iodides that lead to formation of colored product, which require additional purification procedure.

Kamayama, et .al[8] described a method of deprotecting the amino and carboxy by first treating 1 mole of the compound with 1 to 10 moles of phosphorous pentachloride and 1 to 10 moles of a organic base such as pyridine in a chlorinated hydrocarbon solvent such as methylene chloride used in an amount 1 to 50 liter per kg of the compound at –30 to 30°C, to produce a imino-β-lactam compound which is converted to compound 7-AVCA by treating with a phenol selected from phenol, cresol, chlorophenol, methoxyphenol or naphthol used in an amount of 0.5 to 200

kg per kg of the compound in presence of a lower aliphatic alcohol such as methanol used in an amount of 0.01 to 0.05 kg per kilogram of the phenol used at a temperature between 0 to 50°C.

The method described by Kamayama; et .al[8] suffers in that it uses large excess of phosphorous halide, organic base and solvent leading to a large reaction mass.

Lanz, et. al[9] had given a method of preparing compound of formula (**38**) from a carboxy ester of a alkoxycarbonyl or a aryloxycarbonyl protected amino compound (22) in a single step process comprising of treating with a strong acid such as formic acid, trifluoro acetic acid, alkyl or arylsulphonic acid or Lewis acids like Aluminum halides, boron halides, silyl halides in a solvent such as anisole or diethyl ether. The yields of the process vary from 19 to 72%.

The method given by Lanz, et al[9]. has disadvantage in that the yield of Wittig reaction carried out using expensive potassium iodide is low. Also, the method uses large excess of trifluoroacetic acid for deprotection of amino and carboxyl groups.

Gwan son, et al reported[10] the method for deacylation of compound

(**24**) was achieved by treating with PCl_5/CH_2Cl_2 in presence of pyridine.

Deshpande P, et. al [11] have disclosed a process wherein compound of formula (**22**) is treated with triphenyl phosphine in the presence of a solvent such as dichloromethane, acetone or water or a mixture thereof and alkali iodide such as sodium iodide, potassium iodide or lithium iodide; reacting the phosphoranyl intermediate with acetaldehyde using lithium chloride in the presence of a solvent selected from dichloromethane, Dimethyl formamide,isopropyl alcohol, acetone or acetonitrile or a mixture thereof at a temperature range of 0-5°C, deprotecting the

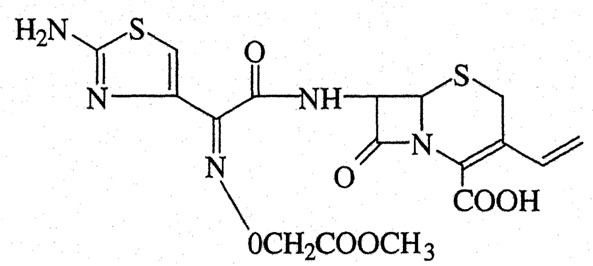
carboxy group with phenol, phenol/TFA,TFA/anisole or formic acid in solvents such as methylene chloride, ethyl acetate, water and like or mixtures thereof; converting the free carboxylic acid to a metal or an amine salt and deacylation with Pen G amidase.

The method reported by Deshpande P, et. al [11] suffers from a limitation in that it uses large excess of solvent and expensive alkali metal iodide that can lead to the formation of colored products that require additional purification.

Methods known in the literature for deprotection of carboxylic ester of β-lactam include, a method using trifluoroacetic acid[12], a method using TFA/anisole[13], a method using formic acid[14]], a method using $AlCl_3$ in the presence of anisole[15] and a method using a phenol[16]

Anuja Chauhan Sisodia, et,al[17] have reported a process for the preparation of Cefixime (**9**) which comprises treating the compound of formula (**54**) with

Na_2CO_3 in DMF and water, which has the following problems: (i) colour and quality are poor, (ii) fails in residual solvent i.e. DMF.



Cefixime ester **54**

Anil Kumar Sharma, et.al[18] have provided a process for the preparation of the compound (**9**) that comprises treating the compound of formula (**54**) in an organic solvent with aqueous solution of Na.$_2$ CO_3 and phase transfer catalyst. However the colour, quality and yield of the product obtained from the bi-phasic reaction mixture is poor.

Kameyama, Yutaka et.al[19] described a method in which hydrolysis step along with deblocking the amino protective group and carboxylate protective group involves the use of phenol and protonic acid. The steps described in the above patents are more complicated and also suffer from low yield and poor quality.

Hideaki Yamanaka, et. al[20] have mentioned various processes for the preparation of Cefixime (**9**). The processes involve the use of column chromatography for purification and poor in yield. Column purification cannot be used in large-scale operations, there by making the process commercially not viable.

Summary, the reported methods for synthesis Cefixime (**9**) suffers from the following disadvantages,

1. Use of alkali metal iodides in Wittig reaction leading to tendency of product to pick up color that require additional purification procedures.
2. Low yields of Wittig products due to the additional steps of isolating the unstable intermediates.
3. Large excess of phosphorous halide and pyridine used in deacylation.
4. Use of large excess of de-esterifying agents such as trifluoroacetic acid.

The conventional procedure for making the compound of formula (**9**) from compound of formula (**22**) involves several intermediate steps of isolating the unstable intermediates in the Wittig reaction, isolating and purifying the ester intermediate leading to lowering of overall yield and longer reaction time.

In the present work the process developed has the following advantages over literature reported processes,

1) Wittig reaction to introduce the vinyl group at C_3 position can be effectively carried out using less expensive sodium bromide in presence of a catalytic amount of a hydrohalic acid.
2) Carrying out the Wittig reaction in one-pot synthesis improved the yield and purity of the Wittig product.
3) In the process use of cheaper reagent like NaBr to prepare the phosphonium salt achieving better quality product substantially free of colored impurities formed in reactions catalyzed by alkali metal iodides.
4) The process avoids usage of excessive mole ratio of reagents like PCl_5, TFA, and large amount of solvent thereby reducing the reaction bulk that facilitates scaling up for an industrial production.
5) The process has effectively less number of steps with an improvement in yield, shortening of reaction time and lowering of labor

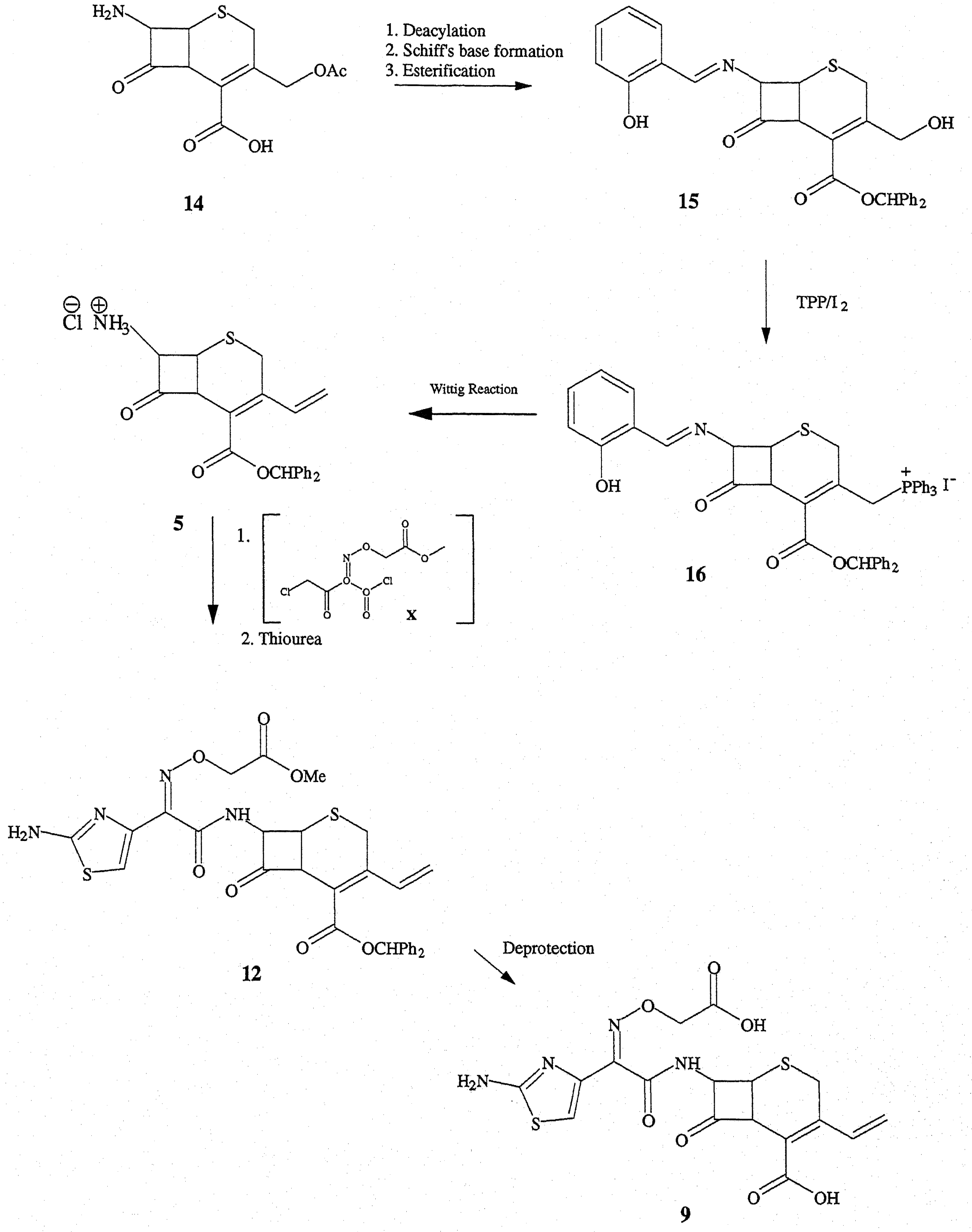
2.3 Objective

An object of the present work is to provide a convenient and cost effective process for the preparation of the Cefixime (**9**), by reducing number of steps and utilizing cheaper raw materials.

The present work also provides a highly selective method for preparation of compound (**9**) in high yield and high purity, substantially free of impurities, which is simple, convenient and cost-effective and more importantly does not suffer from the limitations associated with the prior art methods.

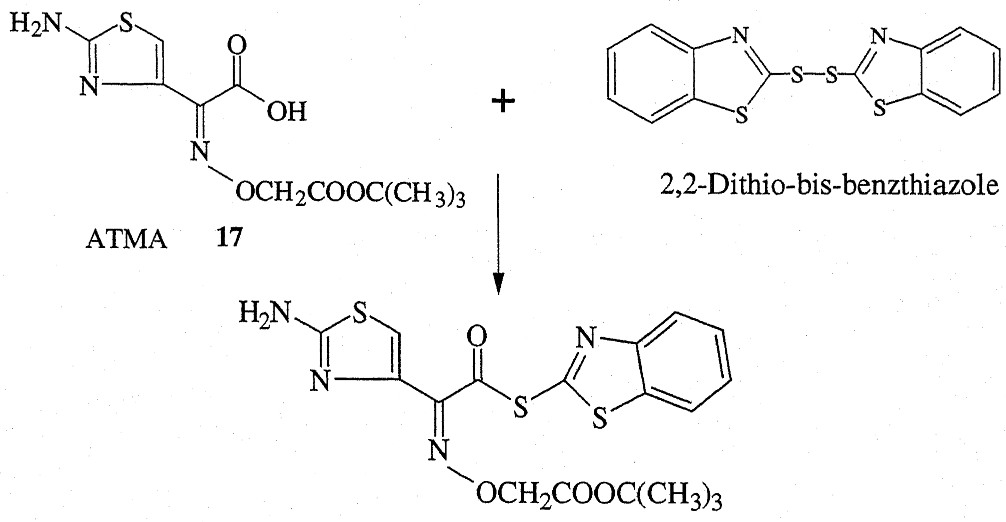
It is yet another object of the present work to identify, synthesize and characterize the impurities formed during the preparation of Cefixime

SCHEME 2.2



1. Deacylation
2. Schiff's base formation
3. Esterification
TPP/I 2
Wittig Reaction
1.
X
2. Thiourea
Deprotection
14
15
16
5
12
9

SCHEME 2.3

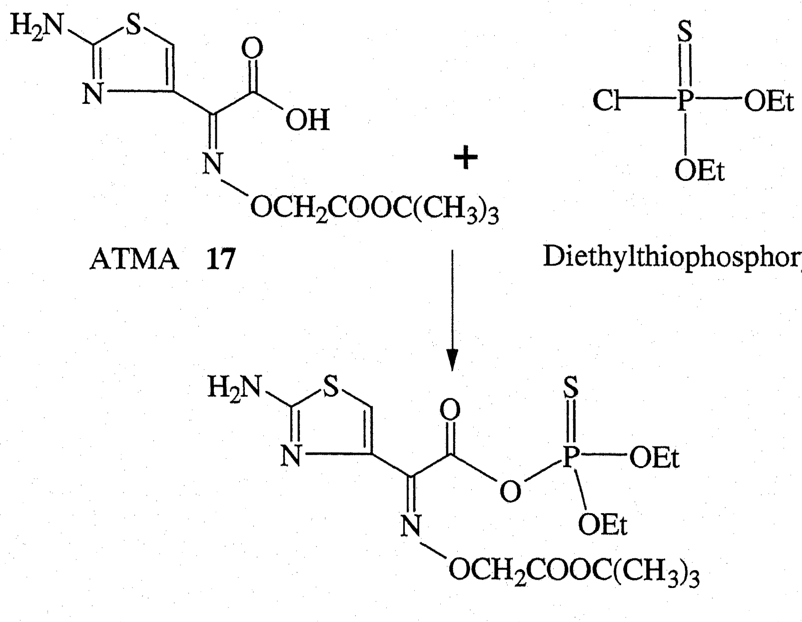


ATMA **17**

2,2-Dithio-bis-benzthiazole **18**

Activated side chain **19**

SCHEME 2.4



ATMA **17**

Diethylthiophosphoryl chloride **20**

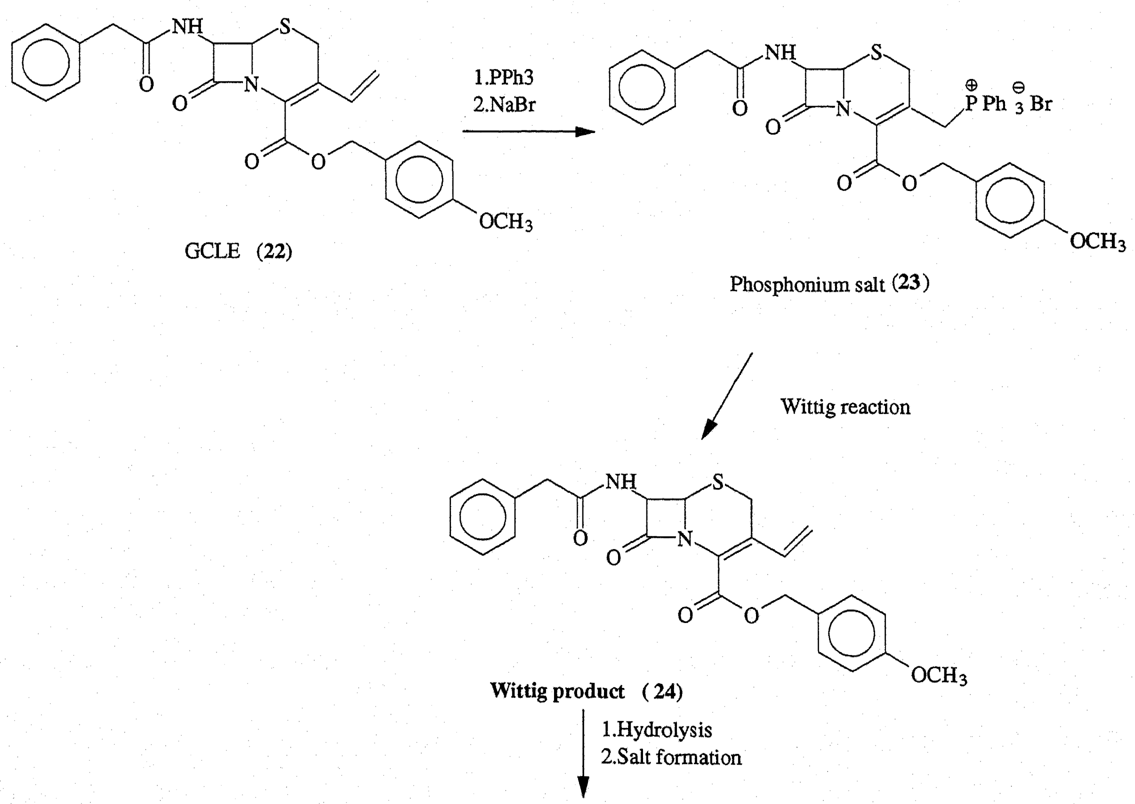
DATMA **21**

2.4.Present work

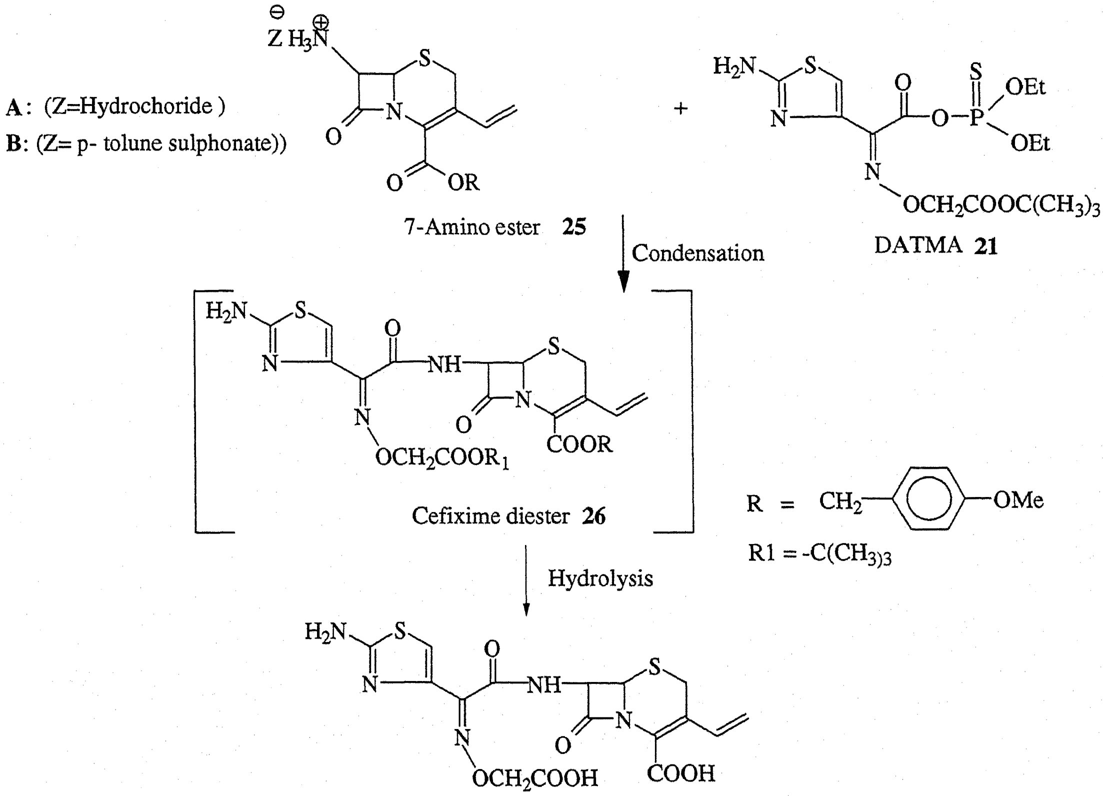
2.4.1 selection of method

Selection of the synthetic scheme was made in parts from the available literature. The Wittig reaction of the protected intermediate (**3**) (Scheme **2.1A**) can be performed analogous to 7-phenylacetamido-3-chloromethyl-3-cephem-4-carboxylalate p-methoxybenzyl ester (GCLE **22**), which also possesses protected carboxyl and amino groups. Subsequent reaction of 3-vinyl GCLE derivative with the activated side chain in analogy with the literature schemes **2.3** and **2.4**. Then the fully protected Cefixime ester can be hydrolyzed to get the required product Cefixime (**9**)as shown in scheme-**2.5**

Scheme -2.5



Scheme -2.5 continued



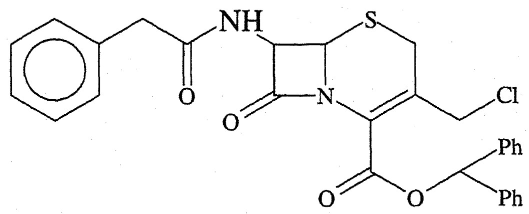
Cefixime (9)

2.4.2 Preparation of Cefixime trihydrate

2.4.2.1 Synhesis of of p-methoxybenzyl-7-phenylacetamido-3-vinyl cephem-4-carboxylate (24) as per scheme –2.5

2.4.2.1.1 Selection of starting material :

On the basis of literature survey we had started our research work to develop cost effective process for the manufacture of Cefixime (**9**) from the intermediate 7-phenyl acetamido-3-chloromethyl-cephem-4-carboxylate-p-methoxybenzyl ester GCLE (**22**) as well as 7-phenylacetamido-3-chloromethyl cephem-4-carboxylate diphenylmethyl ester (GCLH). But GCLE is the choice of intermediate as it is easily available in the market and cheaper than GCLH.



GCLH

Reaction of GCLE, in presence of sodium halide and triphenyl phosphine in the mixture of dimethylformamide and methylene chloride yielded the corresponding phosphonium salts which on reaction with formaldehyde in presence of sodium carbonate furnished 4-p-methoxybenzyl-3-vinyl-7-phenyl-acetamidocephem carboxylate (**24**) hereinafter referred as 3-VBA (Scheme-**2.5**)

2.4.2.1.2 Selection of metal halides & solvents:

In the present work for preparing the phosphonium salt, different metal halides such as sodium iodide, sodium bromide, potassium bromide, potassium iodide, and Lithium bromide were explored in various solvents such as acetone, acetonitrile, dichloromethane, THF, ethyl acetate and mixture of DMF and dichloromethane. Sodium iodide, sodium bromide & lithium bromide, all the three gave the same yield and quality of product (**24**). However, based on the cost consideration and quantity required for the reaction, sodium bromide was considered the metal halide of choice for preparing the phosphonium salts.

Phosphonium salt formation was found to be facile in polar solvent, particularly in DMF with sodium bromide. Other polar solvents such as acetone, acetonitrile did not result in the completion of reaction.

With the view to combine, phosphonium salt formation step and the subsequent step (Wittig reaction), the phosphonium salt formation in the mixture of dichloromethane (DCM) and dimethylformamide (DMF) was explored. Reaction was, therefore, studied in different ratio of DMF/DCM such as 2:1/ 1.5:1.5/ and 0.5:2. The ratio of the solvent DMF: DCM 2:1 & 1.5:1.5, were found to be suitable for phosphonium salt (**23**) formation. Considering the rate and ease of reaction 2:1 ratio of DMF: DCM was fixed for carrying out the reaction .The yield of Wittig product (**24**) is tabularized in (Table-1).

Table – 1: Phosphonium Salt Formation - Wittig Reaction

Solvent Volume		Yield (w/w)
DMF	DCM	
2	1	0.68
1.5	1.5	0.66
0.5	2.0	No Reaction

In order to optimize the ratio of NaBr: TPP ratio, experiments were performed with 1.15:1.25 and 1.15:1.06. Sodium bromide 1.15 mole equivalent of and triphenyl phosphine 1.05 mole equivalent of were found to be ideal for phosphonium salt formation.

2.4.2.1.3 Exploration of Base for Wittig Reaction.

For converting the phosphonium salt to ylide, number of bases such as sodium carbonate, potassium carbonate, sodium hydroxide, sodium bicarbonate, potassium

bicarbonate & triethylamine have been tried. Since sodium carbonate gave comparatively better results, sodium carbonate was selected for Wittig reaction.

2.4.2.1.4 Effect of Temperature on Wittig Reaction.

Effect of temperature on the Wittig reaction was studied by varying the temperature from 0-35°C. There was no effect on the yield and quality of the product (**24**) up to 22-25°C whereas higher temp. (30-35°C) led to the poor yield (Table-2).

Table – 2

Reaction Temp.(°C)	**Yield %(w/w)**	**Remarks**
0-5	0.66	Slow reaction
19-21	0.67	
30-35	0.46	

2.4.2.1.5 Isolation of Product:

For isolating the final product in pure form, dichloromethane was removed at atmospheric pressure and isolation of 3-VBA was studied using solvents like methanol, ethanol and isopropyl alcohol. Methanol was found to be a solvent of choice, since use of ethanol and propanol gave a poor quality of product,

3.4.2.2 Preparation of p-Toluenesulphonate salt of p-methoxyphenyl-7-amino-3-vinylcephem-4-carboxylate (25 B) as per scheme –2. 5 .

p-Toluenesulphonate salt of p-methoxyphenyl-7-amino-3-vinylcephem-4-carboxylate (**25B**), namely 7-AE salt, was prepared by the reaction of p-

methoxybenzyl-7-phenylacetamido-3-vinyl cephem-4-carboxylate with base and PCl_5 to give iminochloride which on treatment with methanol resulted in the hydrochloride salt of p-methoxybenzyl-7-amino-3-vinylcephem-4-carboxylate (**25**-A). This, on treatment with ammonia followed by treatment with p-toluenesulphonic acid in ethyl acetate resulted in the p-toluenesulphonate salt of p-methoxybenzyl-7-amino-3-vinylcephem-4-carboxylate (**25B**, 7-AE Salt).

For the deacylation of p-methoxybenzyl-7-phenyl-acetamido-3-vinylcephem-4-carboxylate, the combination of pyridine / PCl_5 and DMA / PCl_5 were studied. Out of these two combinations, DMA/PCl_5 gave the best results. Hence, the molar ratio of DMA / PCl_5 such as 2.2: 1.7; 2.3:1.and /2.3:1.9/ / were explored (Table-3). Molar ratio of DMA: PCl5 (2.3:1.9) was found to be suitable, which gives better yield of finished product.

Table – 3 : PREPARATION OF 7- AE SALT (25 B)

DMA (Mole)	PCl_5 (Mole)	PCl_5/DMA Ratio	Yield (%)
2.2	1.7	1.29	71.0
2.3	1.8	1.27	72.4
2.3	1.9	1.21	79.6

3.4.2.2.1 Quantity of methanol:

The optimum quantity of methanol 10 mole, 15 moles, 20 moles and 25 moles for hydrolysis of iminochloride was studied i.e. after the reaction of DMA-PCl_5, was studied. 25-mole methanol was found to be appropriate for the cleavage of

iminochloride to amine hydrochloride as it gives better yield of the product (**25 B**, Table-4)

Table – 4

Methanol (Moles)	Yield (%)
10	83
15	84
20	82
25	88

Preparation of of p-methoxybenzyl-7-amino-3-vinyl-3-cephem-4-carboxylate p-toluene sulphonate (25 c-B)

Since the isolated hydrochloride salt of p-methoxybenzyl-7-amino-3-vinylcephem carboxylate (**25-A**) was unstable and picking up the colour, p-TSA salt of 7-AE was prepared after making free base of 7-AE. HCl(**25 A**) with ammonia, followed by treatment with p-TSA.

For preparing the free base of 7-AE.HCl, different bases such as DMA, TEA, NMM, $NaHCO_3$ & ammonia solution were studied. Ammonia was selected as a base of choice, since layer separation during work up was better with ammonia.

2.4.2.2.2 Isolation of (25).

Preparation of p-TSA salt of free base of 7-AE was studied in different mixture of solvents such as Dichloromethane/ Methanol, Dichloromethane /Acetone and

Dichloromethane /Ethyl acetate. The best results were obtained in the mixture of DCM / EtOAc (Table-5)

Table – 5

Isolation solvent	Yield (%)
Dichloromethane/ Methanol	57
Dichloromethane /Acetone	69
Acetone	67
Dichloromethane /Ethyl acetate	89.9

Mode of addition of p-TSA solution in ethyl acetate is critical for isolating the pTSA salt of AE. Addition of free base of 7-AE to the solution of pTSA in ethyl acetate resulted in good yield with fast filtration while the reverse addition resulted in slow filtration of pTSA salt of 7-AE.

The process which emerged after studying all these parameters are given in experimental section.

2.4.2.3: Preparation of Cefixime

Cefixime is prepared from p-Toluenesulphonate salt of p-methoxyphenyl-7-amino-3-vinylcephem-4-carboxylate (**25**) by condensation with DATMA (**21**) to give Cefixime ester (**26**) which on *in-situ* deprotection to afford Cefixime as per scheme –**2.5** .

2.4.2.3.1: Preparation of Cefixime diester

Condensation of 7-AE Salt (**25B**) with 2-(2-amino-4-thiazolyl) 2-[(Z)-t-butoxycarbonylmethoxyimino-acetic acid 2-benzothiazolyl thioester and

diethylthiophosphoryl, 2-amino-4-thiazolyl-2-[(Z)-t-butoxycarbonyl]methoxyimino acetic acid ester (**21**,DATMA) was studied. Although the condensation of 7-AE with former was a neat reaction, removal of 2-mercaptobenzthiazole, a byproduct liberated from the reaction, was found to be difficult. Removal of the same at higher pH resulted in extensive emulsion formation and lead to the decomposition of the condensed product.

Condensation of 7-AE salt with diethylthiophosphoryl 2-amino-4-thiazolyl-2-[(Z)-t-butoxy-carbonyl]methoxyimino acetate (**21**,DATMA) was studied in presence of base.

Although the condensation of 7-AE salt with DATMA in presence of Triethyl amine resulted in the corresponding diester in good yield, but keeping the organic layer for prolonged time (7-8 hrs) resulted in the impurity formation.

To avoid this problem, different bases such as dimethylaniliine and N-methylmorpholine were studied. Both of them gave the same results, but use of dimethylaniline resulted in extensive emulsion formation. Hence, N-methylmorpholine was the choice of base for easier work up as well as minimum degradation of condensed product.

2.4.2.3.2 : Preparation of (6R,7R)-7-[(Z)-2-(2-amino-4-thiazolyl)glyoxylamido]-8-oxo-3-vinyl-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-(Z)-[O-(carboxymethyl)oxime] trihydrate (9,Cefixime trihydrate)

In order to avoid the degradation of Cefixime diester during isolation organic layer of condensed product was *in situ* preceded for deprotection without recourse to isolation of condensed product.

2.4.2.3.3 Exploration of the reagent for deprotection of Cefixime Diester.

Methods known in the literature for deprotection of carboxylic ester of β-lactams include, a method using trifluoroacetic acid[12] a method using TFA/anisole[13] a method using formic acid,[14] a method using $AlCl_3$ in the presence of anisole[15] and a method using a phenol[16].

For converting the Cefixime diester to Cefixime trihydrate, number of reagents were tried such as trifluoro acetic acid/anisole/hydrochloric acid, formic acid/hydrochloric acid), phenol/methanol/acetyl bromide, potassium carbonate/tetra butyl ammonium bromide/DM water, phenol/sulfuric acid and aluminum chloride/anisole in dichloromethane solvent). Aluminum Chloride in presence of anisole and dichloromethane comparatively gave better results in terms of isolation of the product. With use of rest of the reagents, isolation problem was noticed, hence Aluminum Chloride/Anisole reagent was selected for the deprotection of Cefixime diester (Table-6).

TABLE NO. 6

Reagents		Remarks
Trifluoro acetic acid	Anisole	Sticky product
Formic acid	Hydrochloric acid	Sticky product
Acetyl bromide	Methanol/Phenol	Low yield
Aluminum chloride	Anisole	Good yield

In order to optimize the ratio of $AlCl_3$/Anisole, using 2.75 Moles to 4.0 Moles aluminum chloride and with 12 Moles anisole performed reactions. From the experimental data it has been found that 3.55-4.0 moles aluminum chloride and 12 Moles anisole is appropriate for completion of reaction and getting good yield (Table-7).

TABLE NO. 7

$AlCl_3$ (M)	Unreacted intermediate (54)
2.75	6.09%
3.0	4.19%
3.55	0.70%
4	0.10%

2.4.2.3.4: Purification OF Cefixime Trihydrate (Effect of precipitation temperature on quality of Cefixime trihydrate.)

Effect of temperature on the isolation of Cefixime (**9**) was studied varying the temperature from 5 to 35 °C. The product was failed in methanol solubility test at 5°C, higher temp. >30°C resulted in poor description of product. Temperature 24-27°C was found suitable for isolation of Cefixime (Table 8).

TABLE – 8

Temp. (°C)	Solubility in methanol	Description
5	Insoluble	Complies
15	Insoluble	Complies
18	Insoluble	Complies
20	Soluble	Complies
25	Soluble	Complies
27	Soluble	Complies
35	Insoluble	Poor, Not complies

2.4.3 Synthesis of Cefixime impurities.

Cefixime is pharmaceutical product, to meet the quality requirement of pharmacopoeia in terms of minimum impurities, which are mentioned in European pharmacopoeia.[20, 21] The structure of impurities namely D, E, F.and I are given in the European pharmacopoeia[20, 21] the same impurities tried to synthesized in the present work

Beside these known impurities one more impurity observed at RRT (2.08) in our product in the range of 0.05 to 0.4. The mass of the impurity checked by LC mass (680.66), from the mass of the impurity the structure was predicted and here-in referred as impurity LF(**53**). In the present work impurity LF was synthesized and characterized by NMR also confirmed by LC mass.

Number of terms has been commonly used to describe an impurity or impurities[26]

- Intermediate
- Penultimate intermediate
- By-product
- Transformation product
- Interaction product
- Related product
- Degradation products

Some of these terms denote potential sources of impurities

Intermediate : The compound produced during synthesis of the desired material are called intermediates especially if they have been isolated and characterized .The most important criterion is characterization

Penultimate intermediate : As the name suggest, this is the last desired compound in the synthesis chain prior to the production of the final desired compound .

By-product : The unplanned compound produced in the reaction are generally called by products .It may or may not be possible to theorize all of them . Hence ,they present thorny problem to the analytical chemist.

Transformation product : This is a relatively nondescript term which relates to theorized and non theorized product that may be produced in the reaction . Transformation product are very similar to by products, except the term tends to connote that more is known about the reaction products.

Interaction product : This term is slightly more comprehensive and more difficult to evaluate than by-product and transformation product , in that it considers interaction that could occur between various involved chemicals intentionally or unintentionally.

Related products: As mentioned ,the term related product tends to suggest that the impurity is similar to the drug substance and thus tends to play down the negativity frequently attached to the term impurity . These product can have similar chemical structure and potentially similar biological activity .

Degradation product : The compound produced due to decomposition of the material of interest or active ingredient are often referred to as degradation product

Regulatory authorities in each country use their own standards for allowing conductance of clinical studies on new drudges. It should be noted here that efforts are being made to unify these approaches, as exemplified by International Conference on Harmonization (ICH) guidelines

As per FDA requirement if the impurity content greater than 0.1% then it must be characterized

We have directed our research efforts towards making a process for the preparation of Cefixime which overcome not only the drawbacks of the reported methods but which is operationally simple, easily producible on an industrial scale and which give Cefixime trihydrate (**9**) having good quality, stability, solubility and impurity profile.

Mobile phase: (A) 0.05 M ammonium acetate: Methanol (95:5)

pH by 4.2 by H_3PO_4

(B) 0.05 M ammonium acetate: Methanol (50:50)

pH by 3.75 by H_3PO_4

Column: Inertsil ODS 3v 250X4.6 mm, 5μ

Flow: 1.5 ml/min at wavelength 254 nm

Oven temperature: 40 °C.

2.4.3.1 Preparation of impurity D (41)

The impurity D is formed during the synthesis of Cefixime trihydrate in the range of 0.04 to 0.1%. To characterize this impurity it was chemically synthesized as per displayed in the scheme-**2.6A** and **2.6-B**

Synthesis of the Cefixime impurity D (**41**) involved many steps in which synthesis of novel acid 2-(2-amino-4-thiazolyl) 2-[(E)-t-butoxycarbonylmethoxyimino-acetic acid (**37**) is summarized in scheme **2.6-A**.

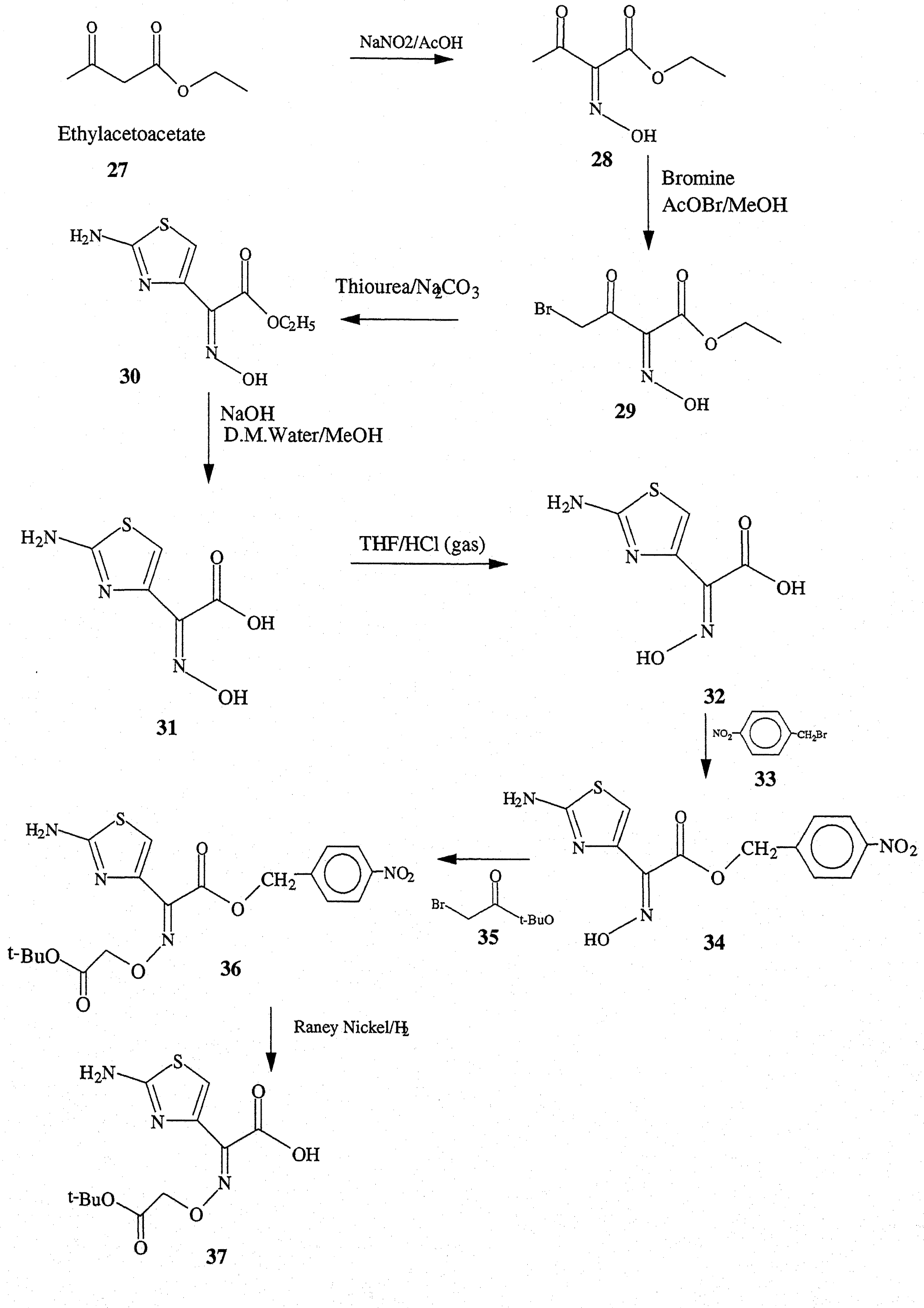
The acid (**37**) was prepared from ethyl acetoacetate (**27**) by treatment with aqueous sodium nitrite in acetic acid to give oxime (**28**), which was brominated.

Cyclisation of bromo derivative (**29**) with thiourea in water by using sodium carbonate to furnished the ester (**30**) The ester obtained was hydrolyzed in aqueous sodium hydroxide solution and isolated at pH 2.5 adjusted by hydrochloric acid to yielded the thiazole acid [22,23] (**31**) which was on isomerisation in hydrochloric acid solution of tetrahydrofuran to afforded anti-isomer of thiazole acid (**32**) .The isomerisation of thiazole acid (**31**) was monitored by HPLC .

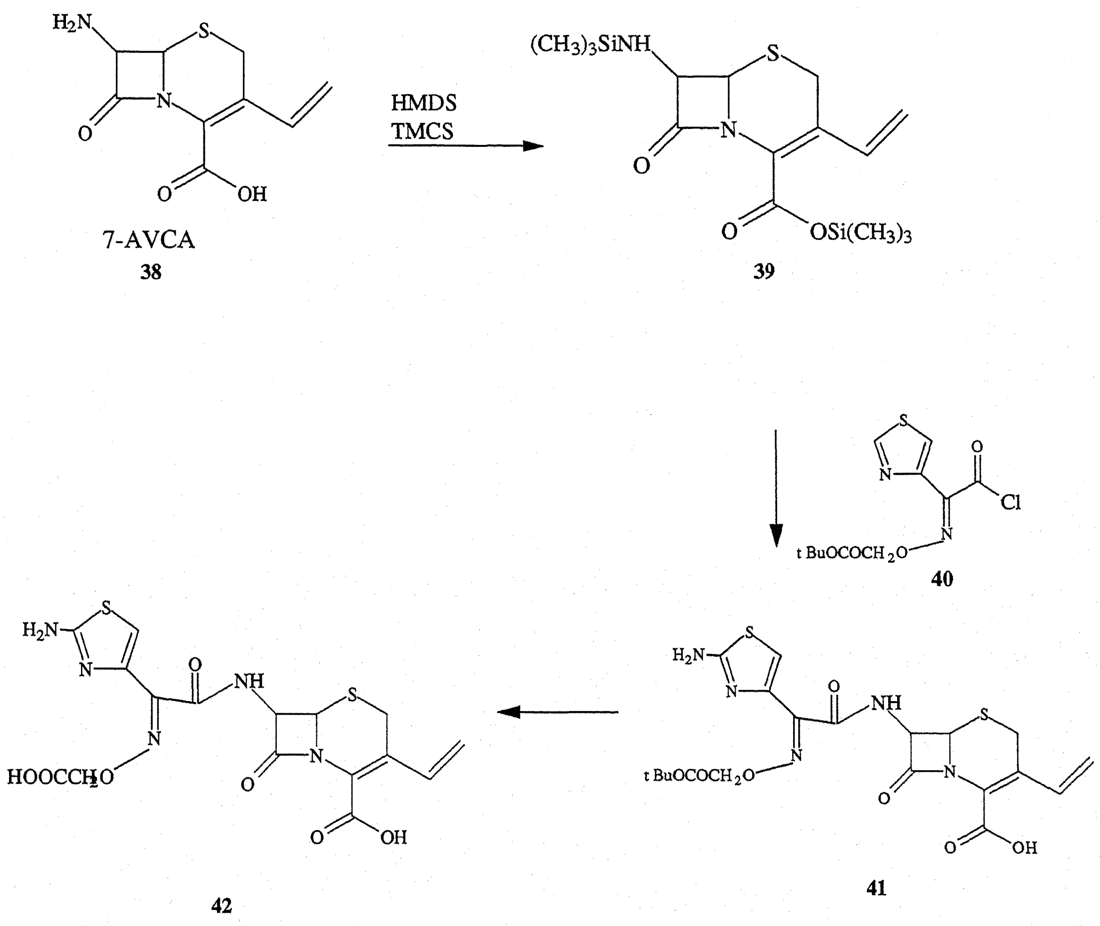
The anti-isomer of thiazole acid was esterified[3] with p-nitrobenzyl bromide in Dimethyl formamide and potassium bicarbonate to obtained the p-nitrobenzyl ester of anti-thiazole acid, which on alkylation with t-butyl bromoacetate to yielded diester (**36**). The p-nitrobenzyl group of the diester (**36**) was selectively cleaved by Raney nickel[24] in aqueous methanolic solution to furnished novel acid 2-(2-amino-4-thiazolyl) 2-[(E)-t-butoxycarbonylmethoxyimino-acetic acid (**37**) which is confirmed by NMR IR and mass.

Impurity D (**42**) was prepared by condensation of the acid chloride (**40**) of (**37**) with disilylated 7-AVCA (**39**) followed by de-esterification of t-butyl group in trifluoroacetic acid [2] to afforded the title impurity (**42**). The detailed procedure is provided in experimental section.

Scheme 2.6 A


NaNO2/AcOH
Ethylacetoacetate
27
28
Bromine
AcOBr/MeOH
Thiourea/Na2CO3
30
29
NaOH
D.M.Water/MeOH
THF/HCl (gas)
31
32
33
34
35
36
Raney Nickel/H2
37

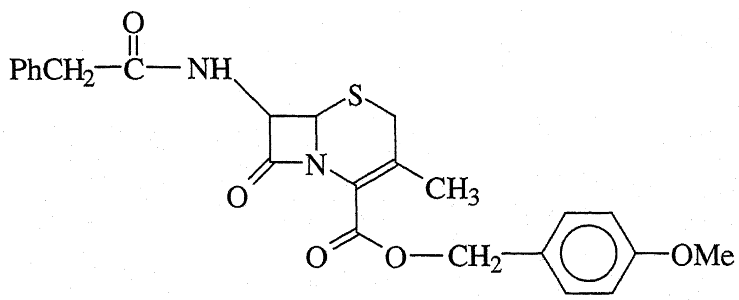
Scheme -2.6 B



2.4.3.2 Preparation of impurity E.

GCLE (**22**) is starting material of Cefixime trihydrate (**9**), contaminated with the compound (**22A**) in the range of 0.3 to 1% which is confirmed by LC mass this compound is chemically inert to the wittig reaction at 3 -position while 3-chloromethyl converted to 3-vinyl, by Wittig reaction. Then both the compounds (**24**)and (**22A)** under go subsequent series of reaction such as deacylation of phenyl acetamide group ,condensation of DATMA (**21**) and deprotection of the

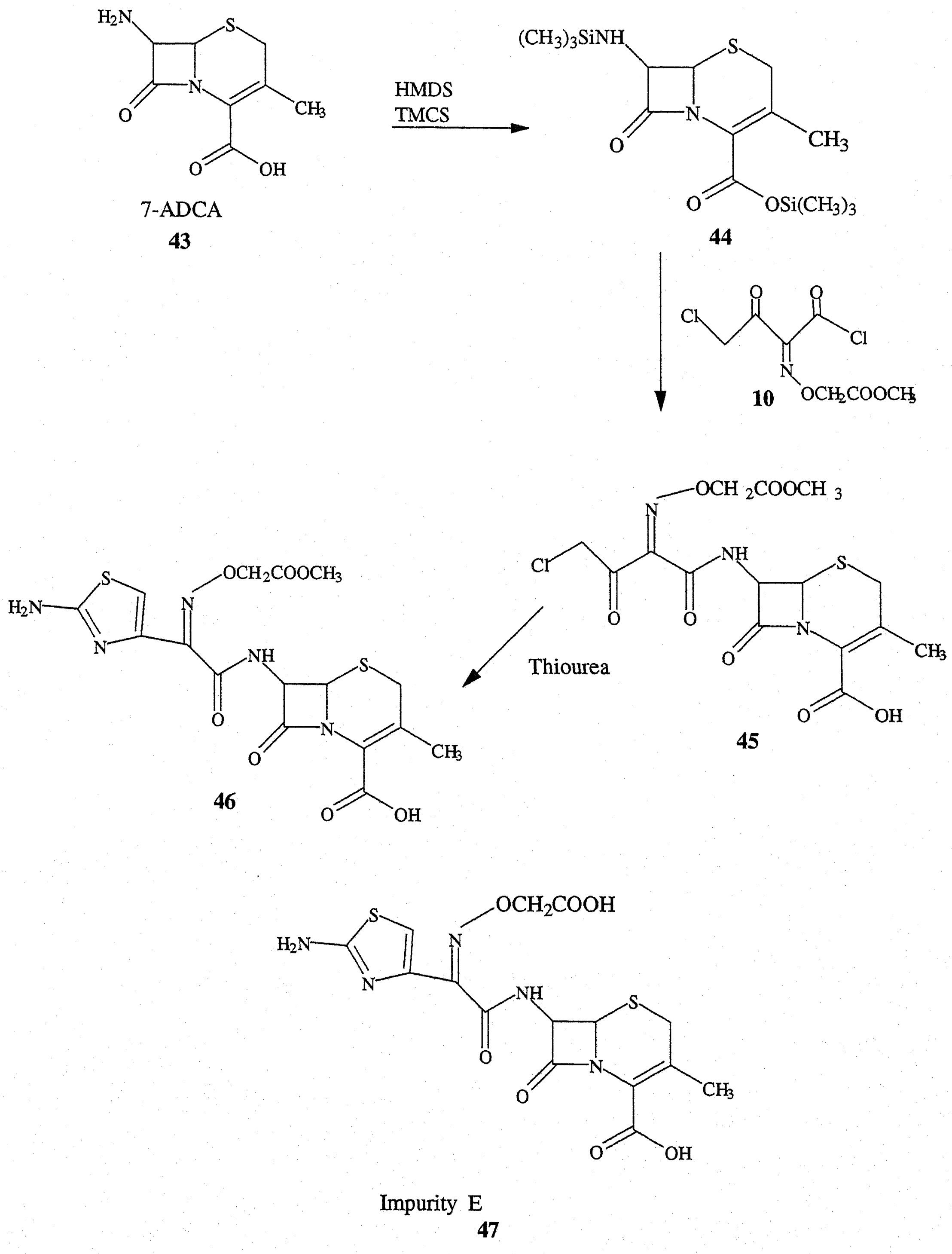
protecting groups simultaneously and therefore impurity E (**47**) exists in as a impurity in the Cefixime .



22 A

Impurity E (**47**) was synthesized from 7–amino-deacetylcephalosporin acid (**43**) by protecting it with silyl and condensation with (**10**) afforded the condensed intermediate (**45**). The condensed product (45) on cyclisation with thio urea yielded Cefixime ester, which is on alkaline hydrolysis furnished the title compound[3] (**47**). The synthetic scheme for the preparation of impurity E is shown in scheme **2.7**. The detailed procedure for synthesis of (47) is explained in experimental section.

Scheme 2.7

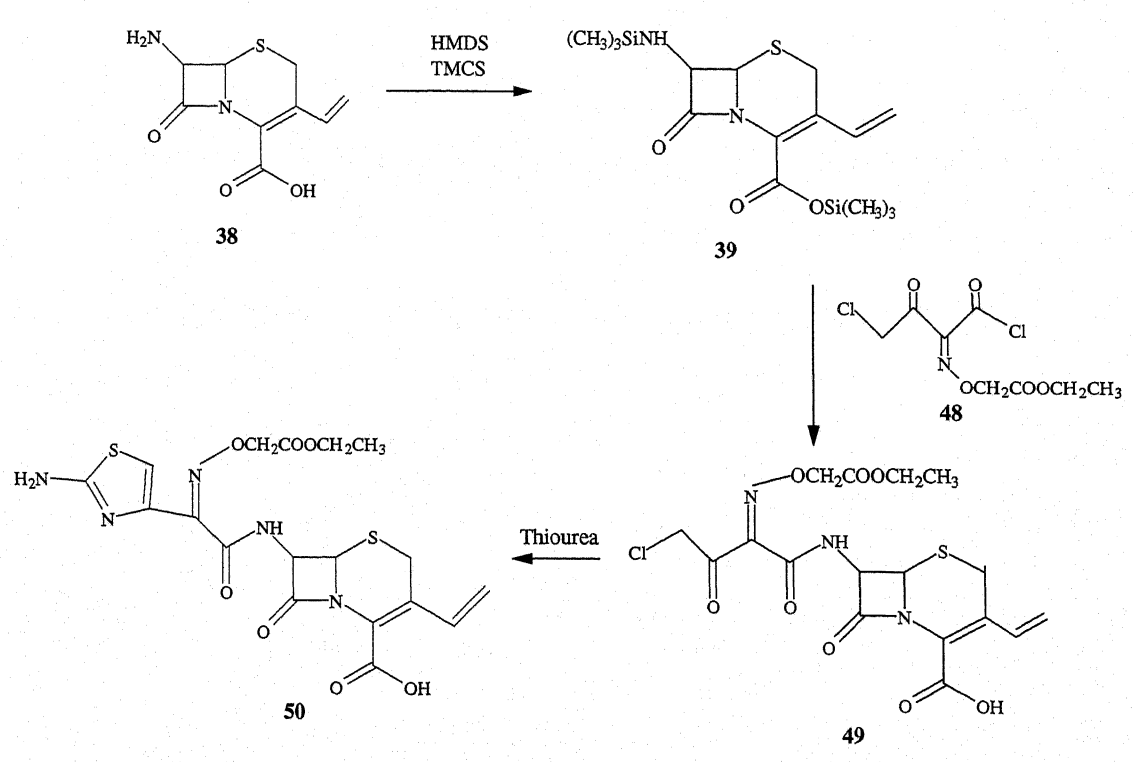


2.4.3.3 Preparation of impurity F.

Impurity F (**50**) was developed in the chemical process, which may be formed during the purification of Cefixime in Ethyl alcohol and water at pH 2.4 to 2.6 at 25 to 27°C. The synthesis of compound (**50**) is summarized in **scheme 2.8**.The starting material (7-AVCA) required for the synthesis of impurity F is commercially available in the market.

7- AVCA was protected with silyl group by treating it with the silylating agent to give di-slylated 7-AVCA which on further reaction with (**48**) afforded the condensed product **(49**) This condensed product was on reaction with thio urea in aqueous media yielded the title impurity F (**50**) [3]. The detailed process is described in experimental section.

Scheme 2.8

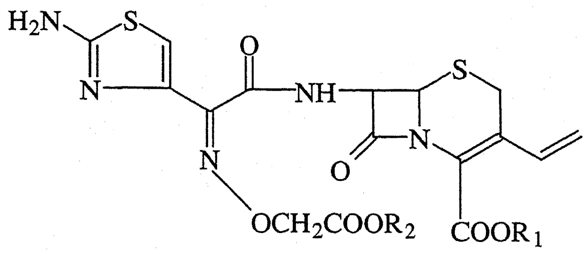


2.4.3.4 Preparation of impurity LF.

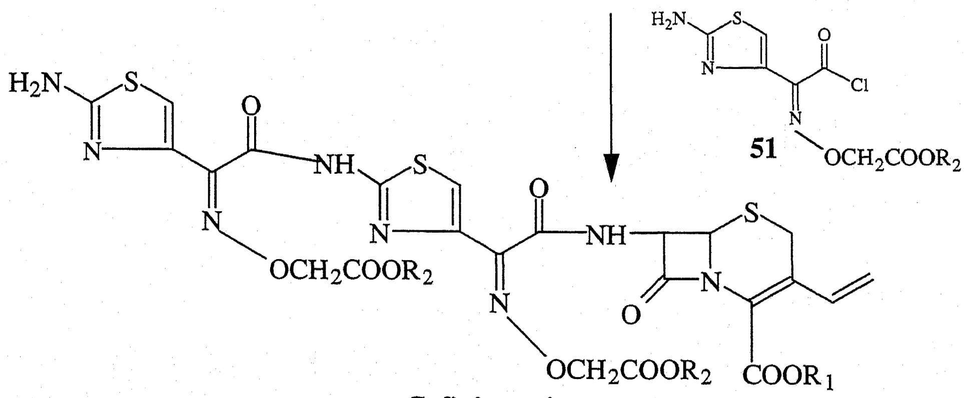
Impurity LF (**53**) is a process impurity generated during reaction of 7-Amino salt (**25B**) with DATMA (**21**) to afforded the mixture of Cefixime diester (**26**) and Cefixime triester (**52**) which on further deprotection in presence of aluminum chloride and anisole yielded the mixture of Cefixime (**9**) and impurity LF(**53**) . This impurity LF is present in the product in the range of 0.05 to 0.2%, which was confirmed by LC mass.

Synthesis of impurity LF (**53**) is summarized in scheme (**2.9**). Cefixime diester (**26**) on reaction with DATMA (**21**) to give the intermediate Cefixime triester (**52**) which on deprotection yielded the title compound (**53**) in impure form. The impure compound (53) was purified by column chromatography using resin XAD –1180 to furnished the pure compound (**53**)[3].

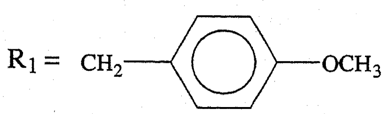
Scheme 2.9



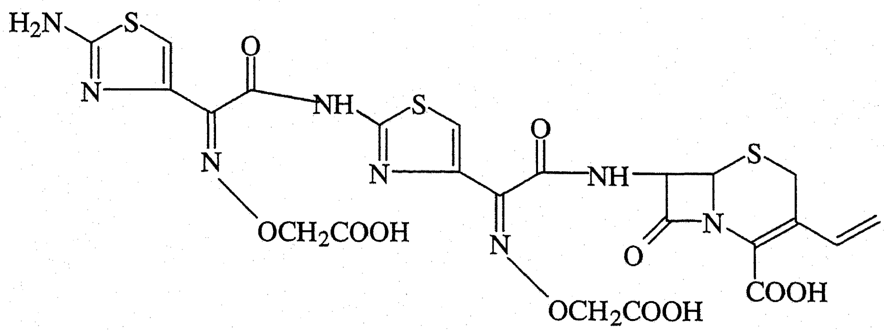
Cefixime diester **26**



Cefixime triester **52**



R_2 = —$C(CH_3)_3$



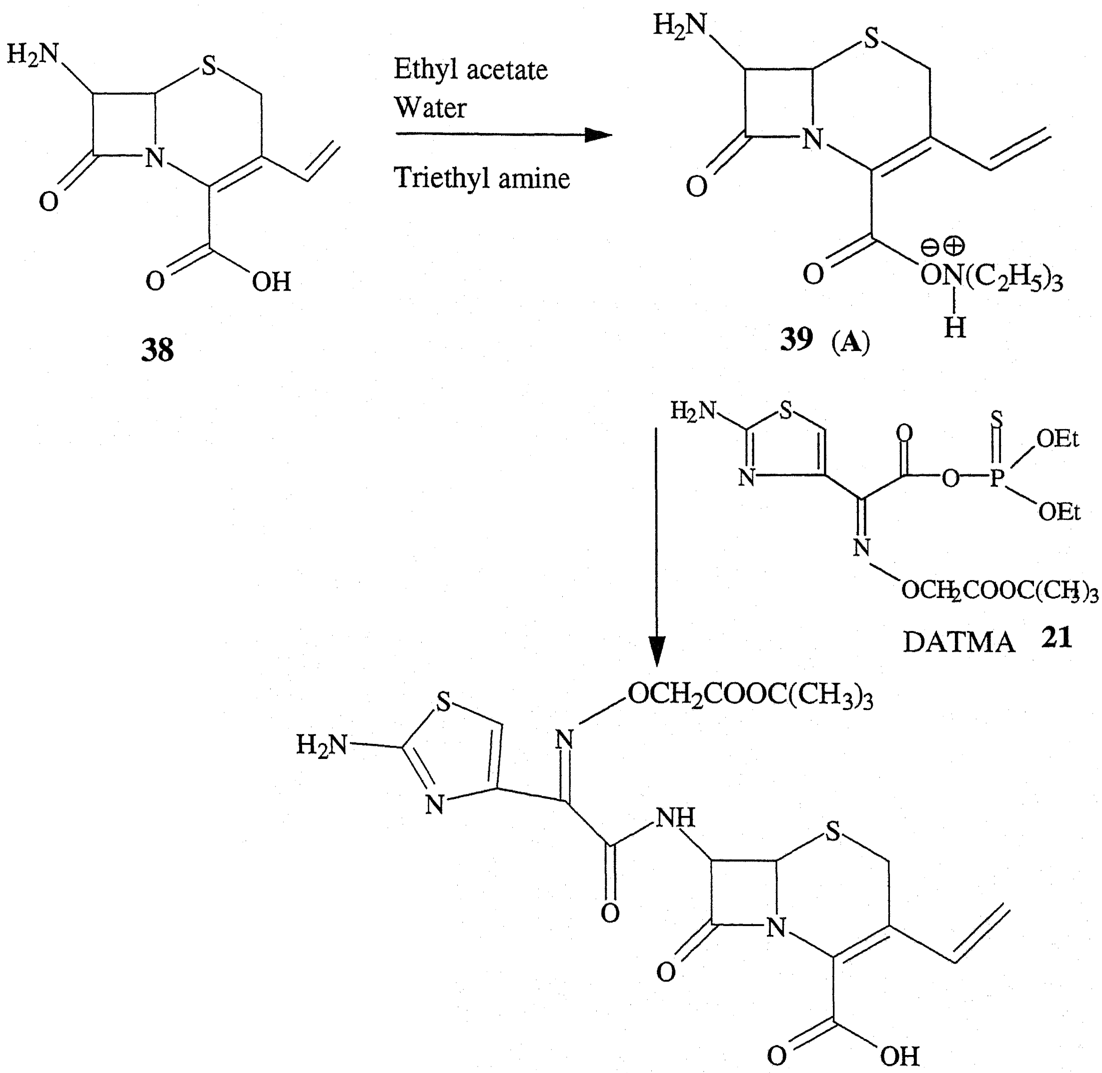
Impurity LF **53**

2.4.3.5 Preparation of impurity I.

Impurity I (**54**) is process impurity generated during the conversion of Cefixime diester (**26**) to Cefixime (**9**). This is an intermediate ester (**54**) found during the HPLC monitoring as unreacted in the range of 0.10to 0.3% in the reaction mixture and carried forward as impurity in Cefixime.

The synthesis of the impurity I is outlined in scheme **2.10.** According to this scheme the 7-AVCA (**38**) on reaction with DATMA (21) in aqueous ethyl acetate at neutral pH maintained by triethyl amine afforded the intermediate triethyl amine salt of (**54**) which on acidification with hydrochloric acid furnished the title impurity I (**54**), [25] which was confirmed by mass and NMR . Detailed process and characterization data is given in e experimental section.

Scheme 2.10



38

39 (A)

Impurity I (54)

2.5 Conclusion.

Cefixime is a commercially valuable and therapeutically useful oral Cephalosporin antibiotic effective in controlling diseases caused by a wide variety of Gram positive and Gram-negative microorganisms.

Because of its therapeutic usefulness and efficient broad spectrum of activity, therefore an improved synthetic process was developed which would resulted in a product with high purity and yield, with minimum level of impurities, preferably absent, coupled with ease of operation and, more importantly, with low production cost.

The present work also provides a highly selective method for preparation of compound **(9)** in high yield and high purity, substantially free of impurities, which is simple, convenient and cost-effective and more importantly does not suffer from the limitations associated with the prior art methods.

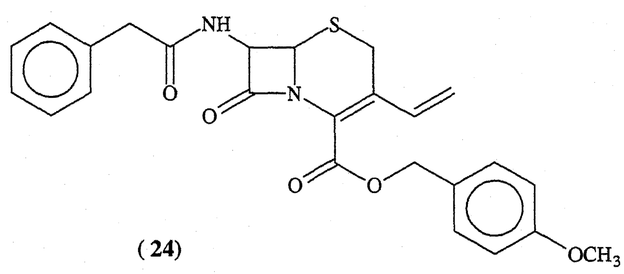
The present work also provided the method for synthesis of the process impurities and their characterization data.

Based on the present work the process was successfully implemented at pilot plant as well as at commercial scale

2.6 Experimental

Preparation of p-methoxybenzyl-7-phenylacetamido-3-vinyl-3-cephem-4-carboxylate (24)

To a solution of p-methoxybenzyl-7-phenylacetamido-3-chloromethyl -3-cephem-4-carboxylate (GCLE,**22**) (10 g,0.0205 mole) in N,N-dimethyl formamide (20 ml) were added hydrogen bromide 48.5% (1.15 g) ,sodium bromide (2.4 g,0.0233 mole) and triphenyl phosphine (5.7 g,0.0217 mole) followed by stirring at 20°C for 2.0 h. The reaction mixture was then added into the mixture of formaldehyde 37%(16.65 g, 0.192 mole) and methylene chloride (50 ml)at 5-15°C Thereto were added solution of sodium carbonate (1.8 g,0.169 mole) in Water (11 ml) followed by stirring at 20°C for 1.0 h .To the reaction mixture was added water (50 ml) and sodium thiosulphate (0.5 g) and pH was adjusted to 4± 0.5 by 10% hydrochloric acid solution .The layer was separated and the organic layer was washed with water (200 ml).The organic layer methylene chloride was distilled out at atmospheric pressure up to three volume and methanol (100 ml)was added . The reaction slurry was cooled to 0-2°C and stirred for 2.0 h. The product was collected by filtration and then dried under vacuum to obtained the title compound **24** (8.0 g,) 84% yield against reported 75% yield.



(24)

IR (KBr) cm^{-1}: 2833-3261,1876,1774,1712,1658,1612,

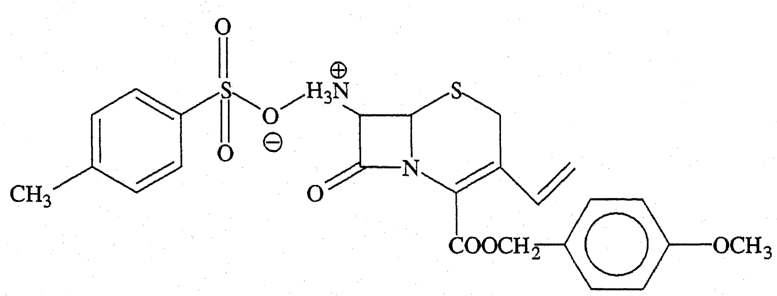
^{1}H NMR Spectrum (DMSO-d_6, d in ppm):

3.50-3.59 m (4H, -S-$\underline{CH_2}$ & -$\underline{CH2}$ pH), 3.73 s (3H, -OCH3), 5.08-5.34 m (1H, vinyl-C=$\underline{CH_2}$ & CO2$\underline{CH2}$ & -CO-CH-$\underline{CH}$), 5.57-5.71 m (1H, - CO$\underline{CH}$-CH), 6.72 – 6.93 m (-$\underline{CH}$=CH_2), 7.2-7.35 m (4H, Benzene-H & p-methoxy benzyl), 9.13-9.17 d (1H, -NH).

Preparation of p-methoxybenzyl-7-amino-3-vinyl-3-cephem-4-carboxylate p-toluene sulphonate (25 B)

To a solution of p-methoxybenzyl-7-phenylacetamido-3-vinyl-3-cephem-4-carboxylate **24** (10.0 g, 0.0215 mole) in dry methylene chloride (120 ml) was maintained at –10 to –15°C while N.N-dimethyl aniline (6.01 g, 0.0495 mole)and phosphorous pentachloride (8.53 g, 0.04091 mole) were added at –50-55°C . The suspension was allowed to warm –35 to –40°C over a period of 60 min .The suspension was cooled to –65 to –70°C and added pre cooled methanol (17.2 g, 0.537 mole) and resulting mixture was stirred at –20 to –25°C for 1.0 h. Transfered the reaction mixture to prechilled D.M. water (100 ml) at –5 °C and stirred for 3.0h at –5°C .The solid was filtered and washed with methylene chloride (40 ml). The wet solid was added into mixture of water (30 ml), methylene chloride (60 ml) at 0-5 °C and pH was adjusted to 5.5 to 6.5 by 5% ammonia solutions. The layer was

separated and extracted the aqueous layer with methylene chloride (40 ml) at 15 to 20°C. Distiled out methylene chloride under vacuum up to three volume. The concentrated reaction mass added into mixture of p-toluene sulphonic acid (5.52 g, 0.029 mole) and ethyl acetate (130 ml) at 10-15°C. Cooled the reaction mixture to 0°C and stirred for 2.0 h. The solid was filtered off, washed with ethyl acetate (20 ml) and dried to give the title product (9.8 g) , yield :88%.



7-Amino ester p-TAS salt **25 B**

IR (KBr) cm^{-1}: 2835-3016,1776,1720,1712,1581,1583,

^{1}H NMR Spectrum (DMSO-d_6, d in ppm):

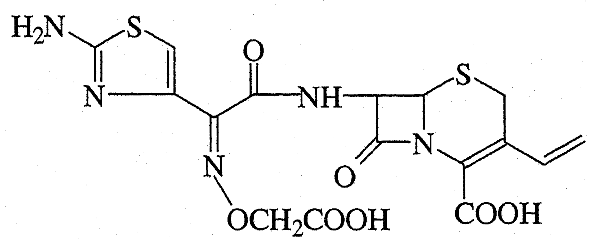
2.26 s (3H, Ph-<u>CH_3</u>), 3.73 s (5H, -OCH3 & -<u>CH2</u> Ph), 3.93-4.02 d (4H, -S-<u>CH_2</u>), 5.10-5.27 m (1H, vinyl-C=<u>CH_2</u> & CO2<u>CH2</u> & -CO-CH-<u>CH</u>), 5.37-5.75 m (1H, - CO<u>CH</u>-CH), 6.82 – 6.96 m (-<u>CH</u>=CH_2), 7.07-7.48 m (8H, Benzene-H & p-methoxy benzyl), 9.02 s (1H, -NH).

Preparation of (6R,7R)-7-[(Z)-2-(2-amino-4-thiazolyl)glyoxylamido]-8-oxo-3-vinyl-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-(Z)-[O-(carboxymethyl)oxime] trihydrate (Cefixime trihydrate (9))

To a suspension of p-methoxybenzyl-7-amino-3-vinyl-3-cephem-4-carboxylate p-toluene sulphonate (**25 B** 10 g, 0.0192 mole) in methylene chloride (100ml) was added Diethyl thiophosphoryl-2-amino-4-thiazolyl-2-[(z)-t-butoxy carbonyl] methoxy

imino acetic, ester [(DATMA **24**) 9.18 g, 0.0203 mole] . To the resultant solution were added N-Methyl morpholine (1.5 g, 0.00148 mole) at –15°C. The temperature was raised to –5°C and stirred for 2 – 4.0 h at same temperature .The reaction mixture was added to water (40 ml) at 10°C The organic layer was separated and washed the aqueous layer with methylene chloride (10 ml). The combined methylene chloride layer was washed with 15% sodium carbonate solution (200ml). Water (20 ml) added to organic layer and pH was adjusted to 3.5 ± 0.5 by 10% hydrochloric acid solution at 10 °C.The layer was separated at 20°C and the aqueous layer was extracted with methylene chloride (10 ml). The combined organic layer was subjected to carbon treatment (1.0 g), filtered and distilled off methylene chloride till 4 volume remains in the flask and drop wise added into the mixture anisole (24.6 g, 227.7mmol) and aluminum chloride (9.72 g,0.0733 mole) in methylene chloride (30 ml) at –10 to –15°C . The reaction mixture was stirred for 2.0 h at same temperature and was added into a mixture of water (80 ml) and 35 %hydrochloric acid (6.5 ml) at 0-5 °C. Methylene chloride (148 ml) added into the reaction mass and stirred for 2.0 h at 0-5°C. The solid was filtered off and washed with methylene chloride (70 ml) and methyl isobutyl ketone (40 ml) followed by ethyl acetate (10 ml). The wet cake (crude Cefixime trihydrate) was taken into water (85 ml) and ethyl acetate (11ml) at 5°C. The pH was adjusted to 5.8 to 6.2 using 4% sodium bicarbonate solution in 2.0 h at 5°C. The reaction mixture was subjected to activated carbon (1.0 g) in presence of ethylene diamine tetra acetic acid (0.10 g). The carbon was filtered off over cellite bed and the filtrate was added into ethyl acetate (60 ml) at 5°C. pH of the mixture was adjusted to 2.5±0.1 by 10%

hydrochloric acid at 5°C in 1.0 h. The temperature was raised to 25°C and stirred for 2.0 h. The solid was filtered, washed with water (20 ml) and ethyl acetate (40 ml). The solid was dried to give the title compound of high purity (7.5 g), yield: **76.5%.** against the reported yield 71%, HPLC purity 99.5%.



Cefixime (9)

R (KBr) cm^{-1}: 3522,3295,2947,1768,1735,,1666,1593,1539,1384,

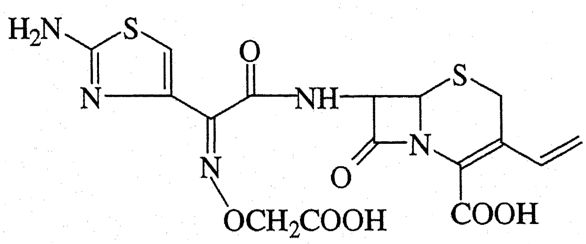
^{1}H NMR Spectrum (DMSO-d_6, d in ppm):

3.53 q (2H, -S-CH_2), 4.60 s (2 H, =N-OCH_2COOH), 5.22 d (1H, vinyl-C=CH_2), 5.29 d (1H, vinyl-C=CH_2), 5.55 d (1H, -CO-CH-CH), 5.78-5.84 q (1H, - CO-CH-CH), 6.72 s (1H, -S-CH= (amino thiazole)), 6.93-6.98 m (1H, -CH=CH_2), 7.29 s (2H, -NH_2) ,9.6 d (1 H, -CO-NH)

Purification of (6R,7R)-7-[(Z)-2-(2-amino-4-thiazolyl)glyoxylamido]-8-oxo-3-vinyl-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-(Z)-[O-(carboxymethyl)oxime] trihydrate (Cefixime trihydrate)

To a suspension of (6R,7R)-7-[(Z)-2-(2-amino-4-thiazolyl)glyoxylamido]-8-oxo-3-vinyl-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-(Z)-[O-(carboxymethyl)oxime] trihydrate (10 g ,0.0197 mole) in water (350 ml) was basified to pH 5.8 to 60 using 2.5% ammonia solution (30 ml) in 2.0 h at 5°C. The clear reaction solution was subjected to carbon (1.0 g) in presence of Ethylene

diamine tetra acetic acid (0.10 g). The carbon was filtered off over cellite bed and the filtrate was added into ethyl alcohol (200 ml) at 5°C. pH of the mixture was adjusted to 3.8±0.1 by 10% hydrochloric acid at 5°C in 1.0 h. The temperature was raised to 27°C and again pH of the mixture was adjusted to 2.5±0.1 by 10% hydrochloric acid in 1.0 h. The solid was cooled to 14±2°C and stirred for 2.0 h at same temperature The solid was filtered, washed with water (50 ml) and ethyl acetate (40 ml). The solid was dried to give the title compound of high purity (9.1 g), Yield :91 %. HPLC purity : 99.7%



Cefixime (9)

R (KBr) cm^{-1}: 3522,3295,2947,1768,1735,,1666,1593,1539,1384,

^{1}H NMR Spectrum (DMSO-d_6, d in ppm):

3.53 q (2H, -S-CH2), 4.60 s (2 H, =N-OCH2COOH), 5.22 d (1H, vinyl-C=CH2), 5.29 d (1H, vinyl-C=CH2), 5.55 d (1H, -CO-CH-CH), 5.78-5.84 q (1H, - CO-CH-CH), 6.72 s (1H, -S-CH= (amino thiazole)), 6.93-6.98 m (1H, -CH=CH2), 7.29 s (2H, -NH2),

9.6 d (1 H, -CO-NH)

Prepration of impurity (6R,7R)-7-{{(E)-2-(2-aminothiazol-4-yl)-2-{(carboxymethoxy)imino}acetylamino}-3-vinyl-8-oxo-5-thia-1-azabicyclo{4.2.0}oct-2-ene-2-carboxylic acid (Imp D)

Step-I : Preparation of Ethyl 2-oxyimino-3-oxo butyrate (28)

To a solution of ethyl acetoacetate (**27**, 100 g, 0.769 mole) in acetic acid (100 ml) was added the aqueous solution of sodium nitrite (60 g, 0.869 mole in 200 ml Water) in 30 min at 0°-5°C and the reaction mass warmed to 25°C. The whole reaction mass was stirred for 1 hr at same temperature. The reaction mixture was added to the ethyl acetate (400 ml) and resulting mixture was stirred for 15 min at 25°C .The ethyl acetate layer was separated and the aqueous layer was extracted with more ethyl acetate (100 ml). The combined ethyl acetate solution was washed with water at pH 7.5 adjusted by 25% potassium bicarbonate solution, dried over anhydrous sodium sulphate and evaporated under reduced pressure to dryness to afforded the oxime of ethyl acetate **(28)** 110 g, Yield: 85% HPLC purity 95%

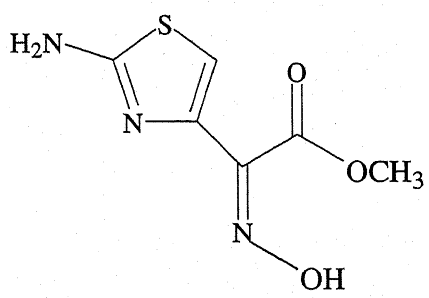
Step-II : Preparation of 4- Bromo ethyl- 2-oxyimino-3-oxo butyrate :

A solution above oxime (**28,** 100 g, 0.625 mole) in ethyl acetate (200 ml) was cooled to –10°C and added Methanol (30 g, 0.937 mole). To this solution acetyl bromide (76.87 g, 0.625 mole) was added slowly in 45 min at 0° to –10°Cthe resulting mixture was stirred for 30 min and bromine (110 g, 0.687 mole) was added over a period of 60 min. The mixture was stirred for 2 hr at –5° to –10°C.The reaction was quenched by 5% aqueous sodium dithionate solution and stirred for 30 min. the ethyl acetate was separated and evaporated to dryness to obtained the bromo derivative of oxime (**29**) 121 g ,Yield 75% HPLC purity 89%

Step-III: Preparation of Ethyl-2-(amino-4-thiazolyl)-2-(Z)hydroxy imino acetate (30) :

To a solution of bromo derivative (**29**,100 g, 0.418 mole) in water (300 ml) was Added thiourea (34.97g, 0.460 moles) followed by sodium acetate (53g, 0.646 moles). The whole reaction mixture was stirred for 2.0 hr. The cyclised solid product (**30**) obtained was & washed water (100 ml). The product was dried under vacuum at 50°C for 5 hr to furnish 80 g, Yield: 94%,

HPLC purity: 98 %



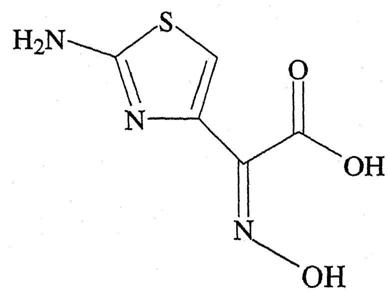
30

[1]H NMR Spectrum (DMSO-d_6, d in ppm):

3.9 s (3H,-<u>OCH_3</u>) 6.6 s (1H, -S-<u>CH</u> = (amino thiazole)).6.9 s (2H,-NH_2),

Step-IV: Preparation 2-(amino-4-thiazolyl)-2-(Z) hydroxy imino acetic acid (31):

To a suspension of the above solid (**30**) (75 g, 0.348 mole) in water (400 ml), solution of sodium hydroxide (32 g 0.802 moles, dissolved in 500 ml water.) was added. Reaction mixture was stirred for 30 min at 0-5°C. After which it was acidified to adjust pH 2.5 to by 5 N Hydrochloric acid solution. Then the product was filtered, washed with water (100 ml) and dried the product under reduced at 50°C for 5 hr to give 56 g 2-amino-α-(Z)-hydrxyimino-4-thiazol acetic acid (**31**),yield : 86% ,HPLC Purity =99.2%.



31

IR (KBr) cm^{-1}: 3283 (OH),3142 (NH_2) ,1672 (C=O)

^1H NMR Spectrum (DMSO-d_6, d in ppm):

6.83 s (1H, -S-CH= (amino thiazole)).7.26 s (2H,-NH_2),

Mass : 188.1 (M + H),.

MSMS : 144.1,126.0

Step V: Preparation 2-(Amino-4-thiazolyl)-2-(E) hydroxy imino acetic acid (32):

The above solid product 2-amino-α- (Z)-hydrxyimino-4-thiazol acetic acid (**31**) (55 g, 0.294 mole) was suspended in tetrahydrofran (200 ml & to this suspension dry Hydrochloric acid gas was bubbled for about 120 min. The resulting solution was stirred for 15 hour at 20-25°C; formation of E-isomer was monitored by HPLC there are two distinct peaks were observed under the same monitoring condition. Then the product was filtered, washed with acetone (100 ml). Dried the product under vacuum at 50°C for 8 hr to furnish 40 g of 2-amino-α-(E)-hydrxyimino-4-thiazol acetic acid (**32**)Yield :80% of HPLC purity 99 %.

Z- Isomer : HPLC retention time 2.90 min

E-isomer: HPLC retention time 3.53min

HPLC condition:

Column: μ bondapak 30 cm

Mobile phase: 0.01M Na_2HPO_4:Acetonitrile (55:45) pH adjusted by H_3PO_4

Flow rate: 1.5 ml/min

Wave length (λ): 235 nm

Column oven temperature: Ambient

Step-VI: Preparation of p-Nitro benzyl- 2-(Amino-4-thiazolyl)-2-(E) hydroxy imino acetate (34):

To a suspension of the above solid 2-amino-α-(E)-hydrxyimino-4-thiazol acetic acid (**32**,50g, 0.316 mole) in N,N-Dimethyl formamide (250 ml) was added p-nitro benzyl bromide (**33**,67g,0.310 mol) & potassium bicarbonate (48 g, 0.48 mole) at 25°-30°C.The whole reaction mixture was stirred for 8 hr at same temperature. Then water (1000 ml) was added to the reaction mass and stirred for 60 min. The solid filtered & washed with water (500 ml) followed methanol (500 ml). The product was dried under reduced pressure at 50°C for 5 hr to afforded 75 g p-nitro benzyl ester (**34**), Yield: 87%, HPLC purity: 85%

Step-VII: **p-Nitro benzyl- 2-(2-Amino-4-thiazolyl)-2-[(E)-(t-butoxycarbonyl methoxy) iminoacetate (36):**

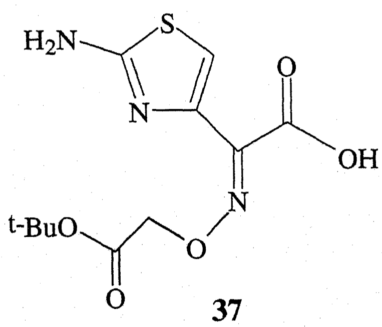
A solution of above p-nitro benzyl ester of thiazole acid (**34**) (50 g, 0.155 mole) in dimethyl formamide (375 ml) was treated with t-butyl bromo acetate (**35**,45.5 g , 0.238 mole) and sodium carbonate (31 g, 0.292 mole) in presence of water (20 ml). . The whole reaction mass was stirred for 8 hr at 40-45°C and filtered, washed with dimethyl formamide (75 ml). The filtrate containing product was added slowly to water (1500 ml) in 2 hr. The solid obtained was stirred for 1 hr and filtered off, washed with water to afforded the diester (**36**) 50 g Yield :74%, HPLC Purity :90%

.Step-VII: 2-(2-Amino-4-thiazolyl)-2-[(E)-(t-butoxycarbonyl) methoxy] imino acetic acid (37) :

A solution of t-butyl ester (**36**, 75 g, 0.172 mole) in methanol (750 ml) was added trimethyl amine (22.5 g, 0.222 mole) and raney nickel (60 g) at 25-30°C. The whole reaction mass was heated to 35-40°C and hydrogen gas bubbled under stirring for 4 hr at same temperature. The reaction mixture was then cooled to 20°-25°C and filtered .The filtrate was evaporated to dryness under reduced pressure. Water (300 ml) was added to the residue and washed with ethyl acetate (500ml) and aqueous solution was acidified to pH 2.5 to 2.7 by 10% hydrochloric acid .The solid obtained was stirred for 1 hr at 25°C and filtered off, washed with water (200 ml) followed by acetone (200 ml).

The solid product was suspended in methanol (150 ml) and heated to refluxed. The slurry was refluxed for 2 hr and cooled to 5°C. The solid was filtered, washed with acetone (100 ml) and dried under reduced pressure to furnish 30g anhydrous 2-(2-

Amino-4-thiazolyl)-2-[(E)-(t-butoxycarbonyl) methoxy] imino acetic acid (**37**),Yield :58%HPLC purity 98%.



37

IR (KBr) cm^{-1}: 3602 (OH),1750 (C=O), 1667 (C=O)

^{1}H NMR Spectrum (DMSO-d_6, d in ppm): **of Z-isomer**

1.2 s (9H, $-(CH_3)_3$), 4.35 s (2H, -O<u>CH</u>$_2$), 6.7 s (1H, -S-CH= (amino thiazole))

7.12 s (2H,$-NH_2$),.

1.42 s (9H, $-(CH_3)_3$), 4.69 s (2H, -O<u>CH</u>$_2$), 7.25 s (2H,$-NH_2$), 7.59 s (1H, -S-CH= (amino thiazole)).

^{1}H NMR Spectrum (DMSO-d_6, d in ppm): **of E-isomer**

1.42 s (9H, $-(CH_3)_3$), 4.69 s (2H, -O<u>CH</u>$_2$), 7.25 s (2H,$-NH_2$), 7.59 s (1H, -S-CH= (amino thiazole)).

Mass : 302.3 (M + H), 324.3 (M + H)

MSMS : 246.2,126.1

Preparation of (6R,7R)-7-{{(E)-2-(2-aminothiazol-4-yl)-2-{(carboxymethoxy)imino}acetylamino}-3-vinyl-8-oxo-5-thia-1-azabicyclo{4.2.0}oct-2-ene-2-carboxylic acid (42):

Solution A:

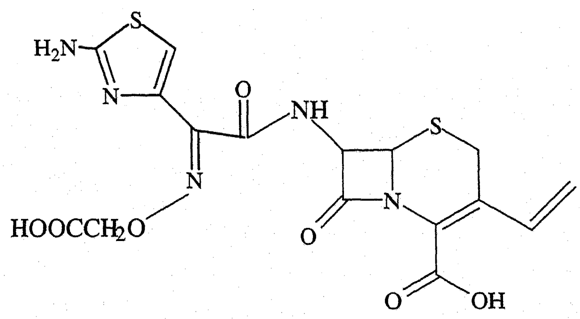
7-Amino-3-vinyl-3-cephem-4-carboxylic acid (**38**,10 g, 0.0442 moles) was suspended in dichloromethane (100 ml) at 25° to 30°C followed by addition of N,O-bis-trimethylsilyl acetamide (0.125 g, 0.0006 mole), trimethylchlorosilane (3.84 g, 0.0353mole) and hexamethyl disilazane (5.71 g, 0. 0.0353 mole). The reaction mass was heated to reflux temperature and refluxed for 2 hours to obtain silylated 7-Amino-3-vinyl-3-cephem-4-carboxylic acid (**39**). The reaction mixture is then cooled to –20°C.

Solution B: Methylene chloride (100 ml) was cooled to 20-25°C, and 2-(2-Amino-4-thiazolyl)-2-[(E)-(t-butoxycarbonyl) methoxy] imino acetic acid (**37,** 13.5 g, 0.044 moles) was added under stirring. The resulting suspension was cooled to –25° to –-30°C and Phosphorous pentachloride (10.2 g 0.0489 moles) is added to it. The mixture was agitated at –20 to -25° for 90 minutes under nitrogen gas purging for removal of hydrochloride gas generateratd during acid chloride formation .The reaction mixture was cooled to –45°C and added a solution of N-methylmorpholine in dichloromethane (0.25 g, 0.0025 mole in 5 ml). The reaction was cooled to –55 °C for condensation.

Solution-C

To a solution of acid chloride of 2-(2-Amino-4-thiazolyl)-2-[(E)-(t-butoxycarbonyl) methoxy] imino acetic acid (**40**) cooled to –55 °C was added with stirring, in the

cooled solution of the di-silylated (**39**). The reaction mixture is stirred at –15° to –20°C for 2 hr. The resulting reaction mass was evaporated under reduced pressure to dryness to obtained an intermediate (**41**) and trifluoroacetic acid (10 ml) was added the residual product (**41**) followed by 35% hydrochloric acid (2 ml) . The solution obtained was stirred for 2 hour at 10°to 15°C. The reaction mixture was added to well cooled water (50 ml) and basified to pH 2.5 to2.7 with 30%aqueous sodium hydroxide solution. The solid obtained was stirred for 2 h at 25°C and filtered off which was dried under reduced pressure at 50°C to get impurity D (**42**) 15 g, Yield: 67%.



42

Molecular formula : $C_{16}H_{15}N_5O_7S_2$

Molecular weight :453.45

Mass : 454.2 (M + H), 472.3 (M + H)

^{1}H NMR Spectrum (DMSO-d$_6$, d in ppm): **Impurity –D (42) (Cefixime E- isomer)**

3.36-3.62 q (2H, -S-<u>CH$_2$</u>), 4.33- s (2 H, =N-O<u>CH$_2$</u>COOH), 5.25 d (1H, vinyl-C=<u>CH$_2$</u>), 5.54 d (1H, -CO-CH-<u>CH</u>),), 5.68 d (1H, -CO-<u>CH</u>-CH), , 6.94-7.28 m (1H, -<u>CH</u>=CH$_2$), 7.44 s (2H, -NH$_2$) , 7.68 s (1H, -S-CH= (amino thiazole)), 9.44 d (1 H, -CO-<u>NH</u>).

^{1}H NMR Spectrum (DMSO-d_6, d in ppm): **Cefixime Z-isomer (9)**

3.53 q (2H, -S-CH$_2$), 4.60 s (2 H, =N-OCH$_2$COOH), 5.22 d (1H, vinyl-C=CH$_2$), 5.29 d (1H, vinyl-C=CH$_2$), 5.55 d (1H, -CO-CH-CH), 5.78-5.84 q (1H, - CO-CH-CH), 6.72 s (1H, -S-CH= (amino thiazole)), 6.93-6.98 m (1H, -CH=CH$_2$), 7.29 s (2H, -NH$_2$), 9.6 d (1 H, -CO-NH).

Prepration of impurity E:

(6R,7R)-7-{{(Z)-2-(2-aminothiazol-4-yl)-2-{(carboxymethoxy)imino}acetylamino}-3-methyl-8-oxo-5-thia-1-azabicyclo{4.2.0}oct-2-ene-2-carboxylic acid (47)

Step A

Methylene chloride (100 ml) was cooled to 20-25°C, and 4-Chloro-2-(Z)-methoxycarbonyl methoxyimino-3-oxo-butyricacid (12.2 g 0.0513 moles) was added under stirring. The resulting solution was cooled to –25° to –-30°C and Phosphorous pentachloride (10.2 g 0.0489 moles) was added to it. The mixture was agitated at –20 to -25° for 90 minutes under nitrogen gas purging for removal of hydrochloride gas generateratd during acid chloride formation .The reaction mixture was cooled to –45°C and added a solution of N-methylmorpholine in dichloromethane (0.25 g, 0.0025 mole in 5 ml). The reaction mass which was a solution of acid chloride (**10**) of 4-Chloro-2- (Z)-methoxycarbonyl methoxyimino-3-oxo-butyricacid was cooled to –55°C for condensation.

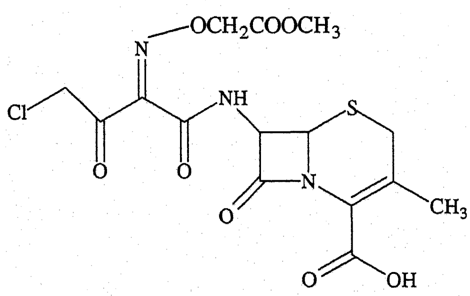
Step B

7-ADCA [(**43**), 10 gm 0.0467 moles] was suspended in dichloromethane (100 ml) at 25° to 30°C followed by addition of N, O-bis-trimethylsilyl acetamide (0.125 g,

0.0006 mole), trimethylchlorosilane (4.15 g, 0.0383 mole) and hexamethyl disilazane (6.03 g, 0. 0.0373 mole). The reaction mass was heated to reflux temperature and refluxed for 2 hours to obtain di-silylated 7–amino-desacetylcephalosporinic acid (**44**) compound. The resulting mixture is then cooled to –20°C.

Step C

To a solution of acid chloride of 4-chloro-2- (Z)-methoxycarbonyl methoxyimino-3-oxo-butyricacid (**10**) product of procedure **2A**, cooled to –55 °C, was added with stirring, in the cooled solution of the disilylated 7-ADCA (**44**) as prepared by procedure **2B**. The reaction mixture was stirred at –15° to –20°C and monitored by HPLC till quantitative conversion to the silylated-condensed product was achieved. The reaction time was about 2 hours. The resulting reaction mass was added to a mixture of 85 ml water and temperature of the reaction mass is raised to 5° to10°C. The slurry obtained was stirred for 2 hour at 5-10° and filtered off. The solid product was washed with dichloromethane (50 ml) followed by Water (100ml) to afforded the intermediate (**45**) 15 g (wet). HPLC Purity 98.11%



45

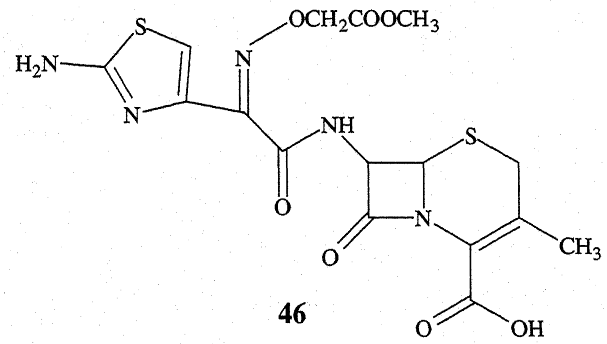
I IR (KBr) cm^{-1}: 3248, 1770 ,1739,1732,1654,1556,1504,1467,1450,1406

^{1}H NMR Spectrum (DMSO-d_6, d in ppm):

1.99 s (3H =C-$\underline{CH_3}$), 3.28-3.37 q (2H, -S-$\underline{CH_2}$), 3.68 s (3H, -CO O <u>CH3),</u> 4.77 s (2 H, =N-O$\underline{CH_2}$COOH), 4.92 S (2H,<u>Cl-CH2-</u>),5.07 d (1H, -CO-CH-<u>CH</u>), 5.67-5.73 q (1H, - CO-<u>CH</u>-CH), , 9.42 d (1H, -<u>NH).</u>

Step D:

A suspension of the above intermediate (**45**) [15 g (wet)] in DM water (100 ml) was treated with thiourea (4.2 g 0.0552 moles) by maintaining the pH of the mixture to 5.5 to 6.0 with 5 % sodium bicarbonate solution for 2 hour under stirring at about 30°Cto get cyclised intermediate (**46**) (of HPLC purity 96.96%)



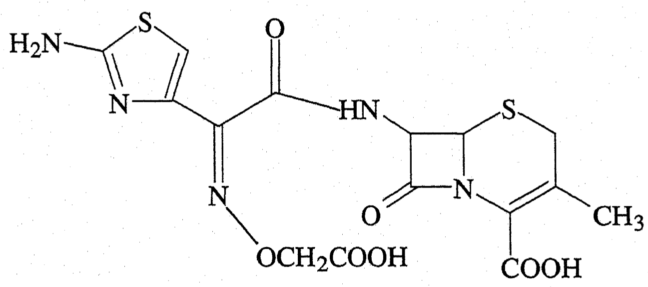
46

IR (KBr) cm^{-1}: 3560,3248,2360,1766, 1660, 1600,1533,1436,1354

^{1}H NMR Spectrum (DMSO-d_6, d in ppm):

2.0 s (3H =C-$\underline{CH_3}$), 3.32-3.37 b (2H, -S-$\underline{CH_2}$), 3.58-3.65 s (3H, <u>OCH3),</u>4.66 s (2 H, =N-O$\underline{CH_2}$COOH), 5.10 d (1H, -CO-CH-<u>CH</u>), 5.65-5.72 q (1H, - CO-<u>CH</u>-CH), 6.78 s (1H, -S-CH= (amino thiazole)), 7.25 s (2H, -NH_2) , 9.51 d (1H, -NH).

The cyclised intermediate (**46**) was then treated with sodium hydroxide (5.04 g 0.126 moles) for 15 min at about 0-5°C. The pH the mixture was adjusted to 5.8 to 6.2 by 10% hydrochloric acid in 2.0 h at 5°C. The reaction mixture was subjected to carbon (1.0 g) in presence of ethylene diamine tetra acetic acid (0.10 g). The carbon was stirred for 30 min and filtered off over cellite bed and the filtrate was added into ethyl acetate (60 ml) at 5°C. pH of the mixture was adjusted to 2.5±0.1 by 10% hydrochloric acid at 5°C in 1.0 h. The temperature was raised to 25°C and stirred for 2.0 h. The solid was filtered, washed with Water (20 ml) and ethyl acetate (40 ml). The solid was dried to give the title Impurity E (**47**) 15g,Yield 75% ,HPLC Purity 98.11%



Impurity -E (47)

Molecular formula : $C_{15}H_{15}N_5O_7S_2$

Molecular weight: 441.44

Mass : 442.4 (M+H)

IR (KBr) cm^{-1}: 3522,3290,1755), 1660, 1647,1577,1558,1527,1429,1409.

^{1}H NMR Spectrum (DMSO-d_6, d in ppm):

2.03 s (3H =C-$\underline{CH_3}$), 3.28-3.72 q (2H, -S-$\underline{CH_2}$), 4.59 s (2 H, =N-O$\underline{CH_2}$COOH), 5.11 d (1H, -CO-CH-$\underline{CH}$), 5.68-5.75 q (1H, - CO-$\underline{CH}$-CH), 6.80 s (1H, -S-CH= (amino thiazole)), 7.27 s (2H, -NH_2), 9.51 d (1H, -NH).

Preparation of Impurity F:

(6R, 7R)-7-[[(Z-2- (2-aminothiazol-4-yl)-2-[(2-ethoxy) imino] acetyl] amino]-3-ethenyl-8-oxo-5-thia-1-azadicyclo [4.2.0] oct-2-ene-2-carboxylic acid (50)

Step:1 Preparation of 7β-(4-chloro-2-methoxycarbonyl ethoxyimino-3-oxobutyramido)-3-vinyl-3-cephem-4-carboxylic acid (**49**)

Solution A:

7-Amino-3-vinyl-3-cephem-4-carboxylic acid (**38** , 10 g, 0.0442 moles) was suspended in dichloromethane (100 ml) at 25° to 30°C followed by addition of N,O-bis-trimethylsilyl acetamide (0.125 g, 0.0006 mole), trimethylchlorosilane (3.84 g, 0.0353mole) and hexamethyl disilazane (5.71 g, 0. 0.0353 mole). The reaction mass was heated to reflux temperature and refluxed for 2 hours to obtain silylated 7-Amino-3-vinyl-3-cephem-4-carboxylic acid (**39**). The resulting mixture is then cooled to –20°C.

Solution B:

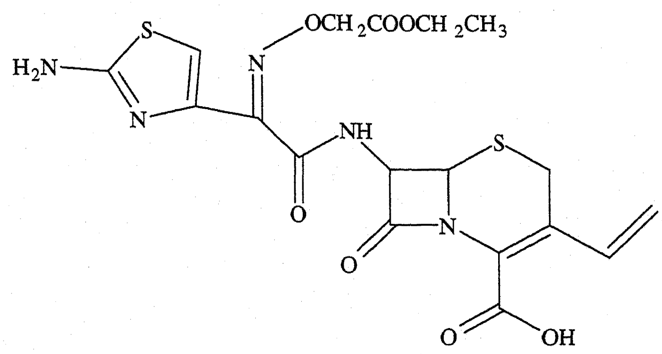
Methylene dichloride (100 ml) was cooled to 20-25°C, and 4-chloro-2-(Z)-methoxycarbonyl ethoxyimino-3-oxo-butyric acid [**48** ,(11.68 g 0.0464 moles)] was added under stirring. The resulting solution was cooled to –25° to —30°C and Phosphorous pentachloride (10.2 g 0.0489 moles) is added to it. The mixture was agitated at –20 to -25° for 90 minutes under nitrogen gas purging for removal of hydrochloride gas generateratd during acid chloride formation .The reaction mixture

was cooled to –45°C and added a solution of N-methylmorpholine in dichloromethane (0.25 g, 0.0025 mole in 5 ml). The reaction mass which was a solution of acid chloride of 4-Chloro-2- (Z)-methoxycarbonyl ethoxyimino-3-oxo-butyricacid (**48**) was cooled to –55 °C for condensation.

To a solution of acid chloride of 4-Chloro-2-(Z)- methoxycarbonyl ethoxyimino-3-oxo-butyricacid (**48**) product of procedure **2A**, cooled to –55 °C, was added with stirring, in the cooled solution of the disilylated 7-AVCA as prepared by procedure **2B**. The reaction mixture was stirred at –15° to –20°C and monitored by HPLC till quantitative conversion to the silylated-condensed product was achieved. The reaction time was about 2 hours. The resulting reaction mass was added to a mixture of 85 ml water and temperature of the reaction mass was raised to 5° to10°C. The slurry obtained was stirred for 2 hour at 5-10° and filtered off. The solid product was washed with dichloromethane (50 ml) followed Water (100ml) to afforded the intermediate (**49**) 15 g (wet).

Step 2

A suspension of the above intermediate (**49**) [15 g (wet)] in DM water (100 ml) was treated with thiourea (5.04 g 0.0663 moles) by maintaining the pH of the mixture to 5.5 to 6.0 with 5 % sodium bicarbonate solution for 2 hour under stirring at about 30°C to get cyclised intermediate(**50**) then mixture was acidified to pH 2.5±0.1 by 10% hydrochloric acid at 5°C in 1.0 h. The temperature was raised to 25°C and stirred for 2.0 h. The solid was filtered, washed with Water (20 ml) and ethyl acetate (40 ml) . The solid was dried to give the title Impurity F(**50**) 10 g .



50

Molecular formula : $C_{18}H_{19}N_5O_7S_2$

Molecular weight: 481.45

Mass : 482.5 (M+H),504.6 (M+H)

^{1}H NMR Spectrum (DMSO-d_6, d in ppm):

1.16-1.23 t (3H, -CH2CH3), 3.49-3.73 q (2H, -S-CH2),4.08-4.19 m (3H, -OCH2CH3) 4.60 s (2 H, =N-OCH2COOH), 5.21 d (1H, vinyl-C=CH2), 5.29 d (1H, vinyl-C=CH2), 5.55 d (1H, -CO-CH-CH), 5.78-5.84 q (1H, - CO-CH-CH), 6.79 s (1H, -S-CH= (amino thiazole)), 6.93-6.98 m (1H, -CH=CH2), 7.26 s (2H, -NH2) , 9.6 d (1H, -NH).

Preparation of Impurity LF:

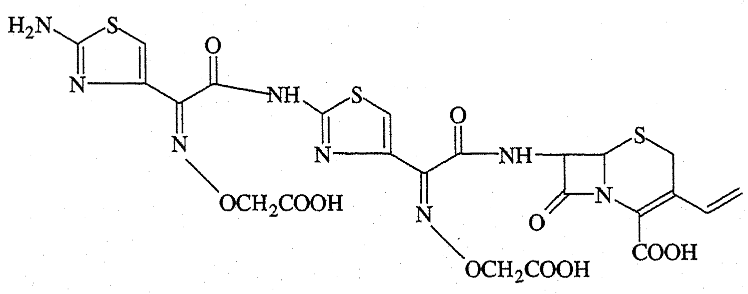
Preparation of Impurity- (6R)-6-((2Z)-2-{2-[(2Z)-2-(2-amino (1,3-thiazol-4-yl)}-3-aza -3-(carboxymethoxy) prop-2-enoylamino)-5-oxo-3-vinyl-2H,6h,6aH-azetidino[2,1-b]1,3-thiazine-4-carboxylic acid (LF)

Methylene chloride (100 ml) was cooled to 20-25°C, and 2-(2-amino-4-thiazolyl) 2-[(Z)-t-butoxycarbonylmethoxyimino-acetic acid **(17)** (5.26 g 0.017 moles) was added under stirring. The resulting solution was cooled to –25° to —30°C and Phosphorous pentachloride (10.2 g 0.0489 moles) is added to it. The mixture was agitated at —5 to -10° for 90 minutes under nitrogen gas purging for removal of

hydrochloride gas generateratd during acid chloride formation .The acid chloride solution (**51**) of 2-(2-amino-4-thiazolyl) 2-[(Z)-t-butoxycarbonylmethoxyimino-acetic acid was cooled to –15°C and added to a solution of Cefixime ester in dichloromethane (10 g, 0.0158 mole in 50 ml). The temperature was raised to –25°C and stirred for 8 h at same temperature. The reaction was monitored by TLC (Mobile phase Chloroform: Methanol, 7:3). The reaction mixture was added to Water (40 ml) at 25°C, and the organic layer was separated. The aqueous layer was washed with methylene chloride (20 ml). The combined methylene chloride layer was washed with 15% sodium carbonate solution (100ml). Water (20 ml) added to organic layer and pH was adjusted to 3.5 ± 0.5 by 10% hydrochloric acid solution at 10 °C.The layer was separated at 25°C and the aqueous layer was extracted with methylene chloride (10 ml).

Distilled off methylene chloride layer up to 4 volume to afforded the Cefixime triester (**52**) and drop wise added into the mixture anisole (24.6 g, 227.7mmol) and aluminum chloride (10.45 g, 0.788 mole) in methylene chloride (30 ml) at 0 to 05°C. The reaction mixture was stirred for 3.0 h at same temperature and was added into mixture of Water (80 ml) and 35 %hydrochloric acid (6.5 ml) at 0-5 °C. Added methylene chloride (148 ml) into the reaction mass and stirred for 2.0 h at 0-5°C. The solid was filtered off and washed with methylene chloride (70 ml) the wet cake (crude impurity LF) was taken into Water (85 ml) and ethyl acetate (11ml) at 5°C. The pH was adjusted to 5.8 to 6.2 using 25% potassium bicarbonate solution in 1.0 h at 15°C the resulting solution was passed through resin XAD -1180 and the elute of the pure fraction was acidified to pH 2.5±0.1 by 10% hydrochloric acid at 25°C in

1.0 h. The solid was stirred for 2.0 h. and filtered, washed with Water (20 ml) and ethyl acetate (40 ml). The solid was dried to give the title compound (**53**) g.



Impurity LF **53**

Molecular formula : $C_{23}H_{20}N_8O_{11}S_3$

Molecular weight: 680.66

Mass : 681.6 (M+H),703.6 (M+H)

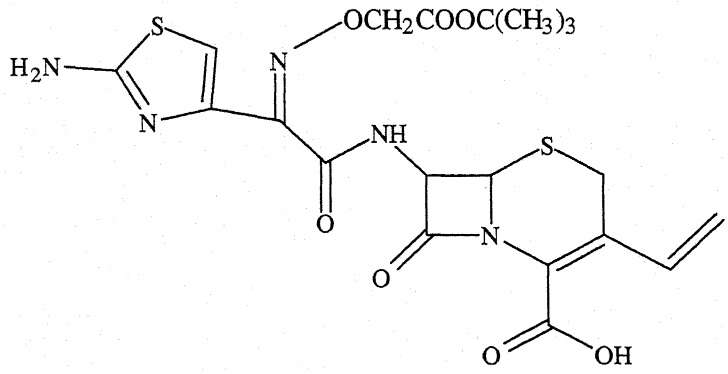
^{1}H NMR Spectrum (DMSO-d_6, d in ppm):

3.54 q (2H, -S-CH₂), 4.55 s (2 H, =N-OCH₂COOH), 5.21 d (1H, vinyl-C=CH₂), 5.27 d (1H, vinyl-C=CH₂), 5.56 d (1H, -CO-CH-CH), 5.73-5.79 q (1H, - CO-CH-CH), 6.76-6.90 m (1H, -CH=CH₂),7.42 (1H, -S-CH= (amino thiazole)), 9.63 s (2H, -NH₂), 13.16 s (1H, -COOH).

Preparation of (6R, 7R)-7-[[(Z-2- (2-aminothiazol-4-yl)-2-[(2-t-butoxy) imino] acetyl] amino]-3-ethenyl-8-oxo-5-thia-1-azadicyclo [4.2.0] oct-2-ene-2-carboxylic acid (Impurity I)

7-Amino-3-vinyl-3-cephem-4-carboxylic acid (**38**) (10 g, 0.0442 moles) was suspended in mixture ethyl acetate (100 ml) and water (30 ml) at 25° to 27°C the mixture was cooled to 0°C and triethyl amine (4,69 g, 0.0469 mole) was added followed by addition of DATMA [**21**, (21.95 g, 0.0484 moles)] at 0°C. The reaction

mixture is stirred at 0 to –5°C for 2 hour. Water (80 ml) was added to the reaction mixture and aqueous layer was separated. The aqueous solution was acidified to pH 2.5±0.1 by 10% hydrochloric acid at 15°C in 1.0 h. The temperature was raised to 25°C and stirred for 1.0 h. The solid was filtered, washed with Water (50 ml) and ethyl acetate (50 ml). The solid was dried to give the title Impurity I (**54**) 15 g.



Impurity I (**54**)

Molecular formula : $C_{20}H_{23}N_5O_7S_2$

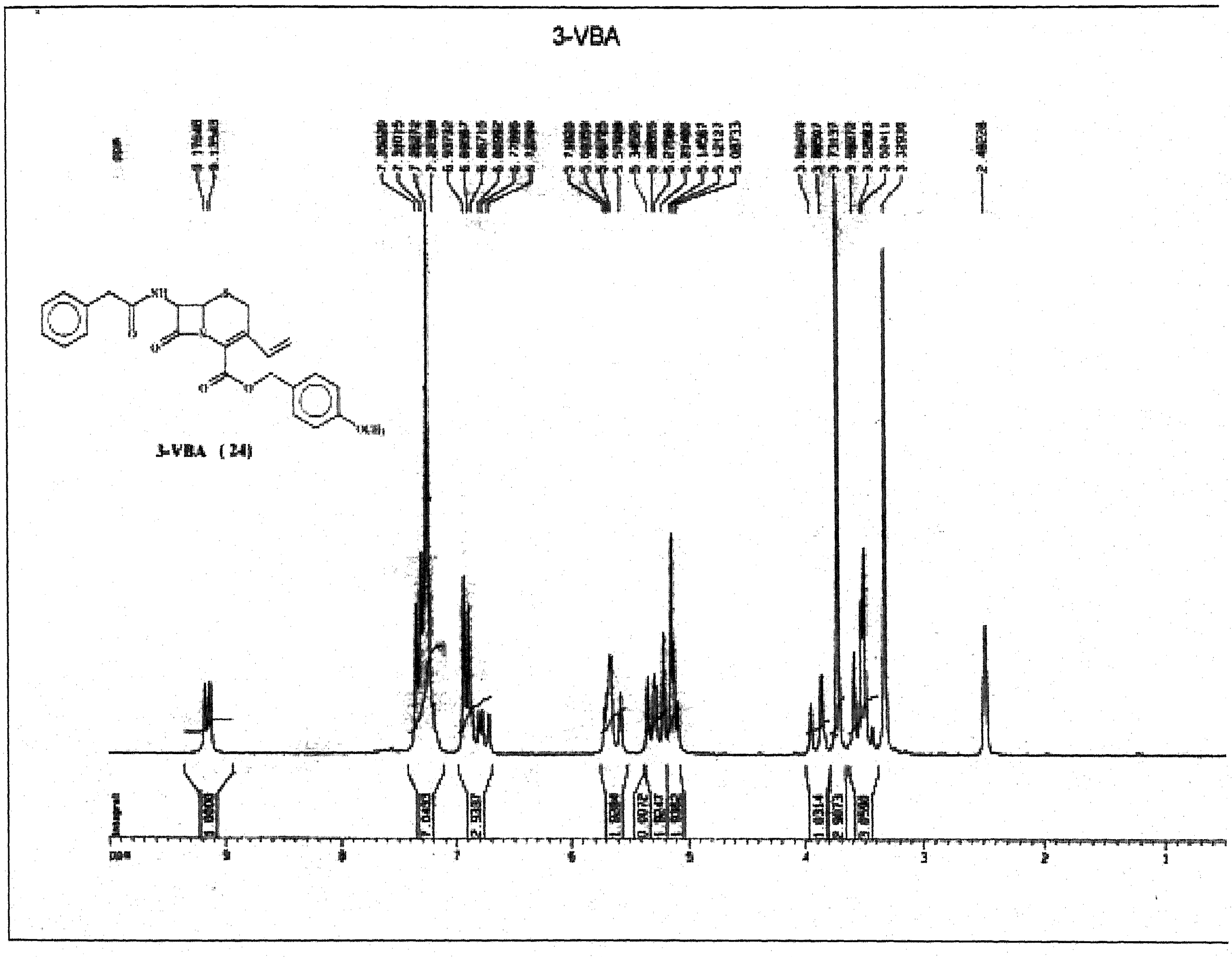
Molecular weight: 509.56

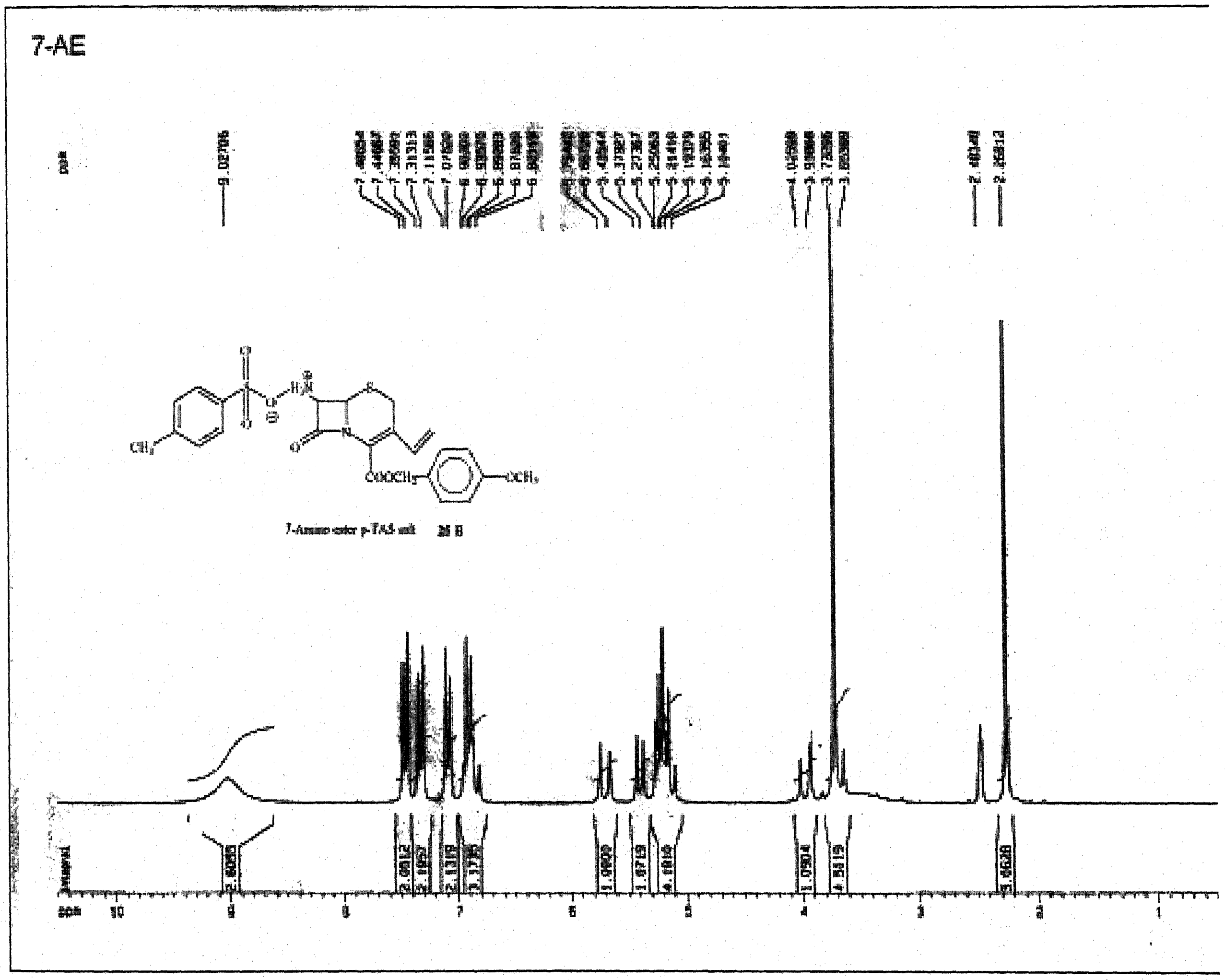
Mass : 510.3 (M+H),532.4 (M+H)

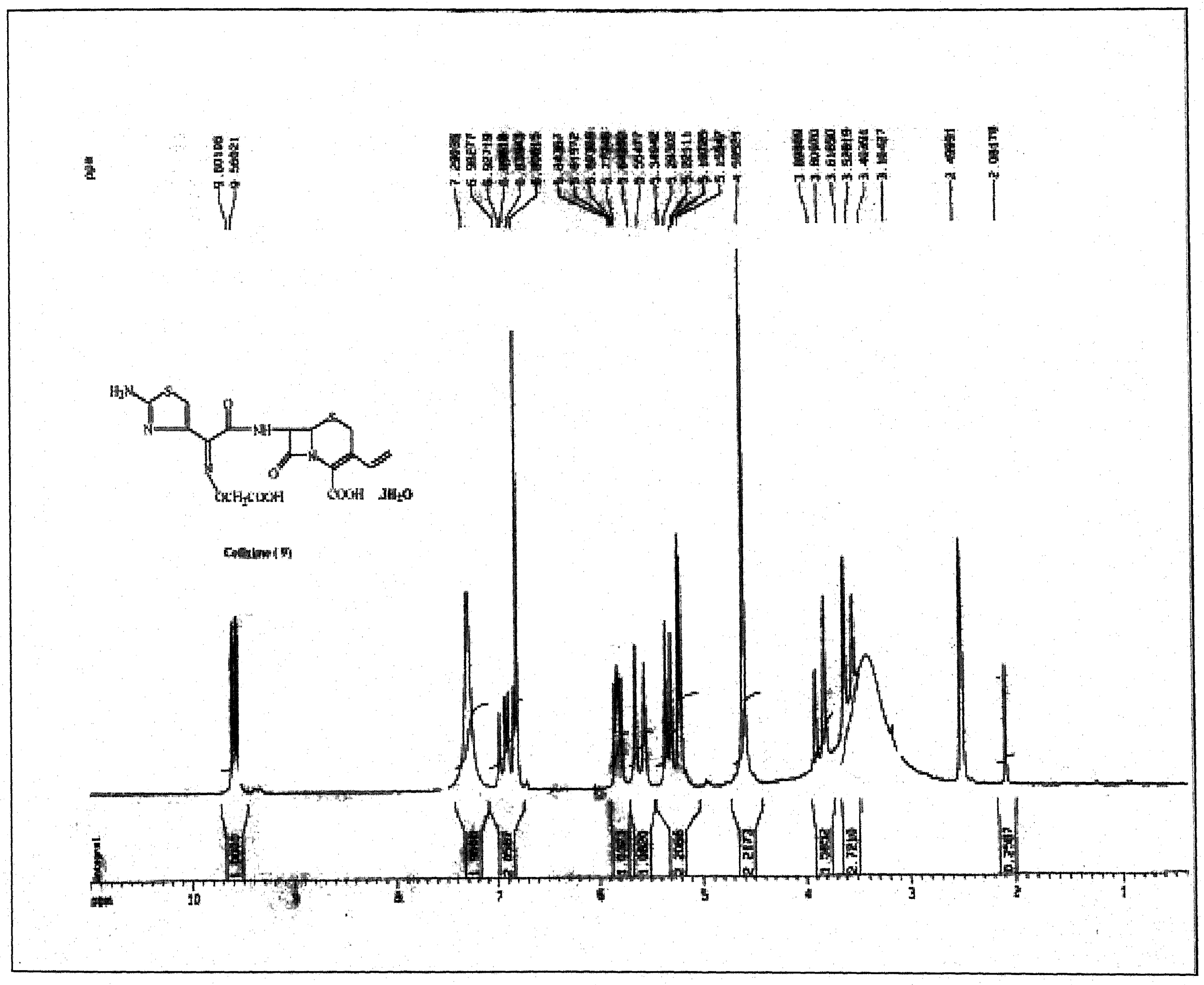
IR (KBr) cm^{-1}: 3316,2974,1764, 1680, 1618,1533,1456,1368,1246,1156.

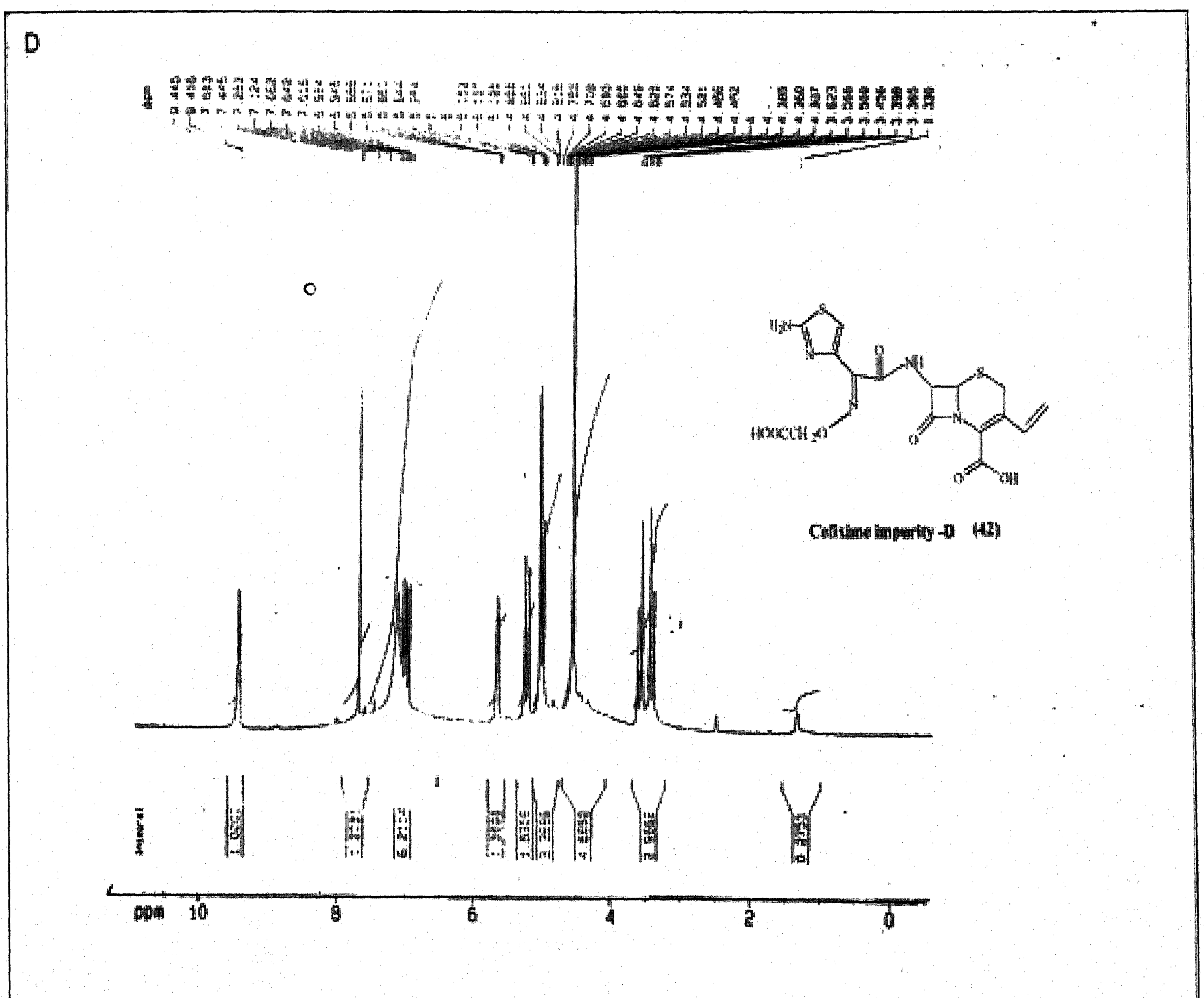
^{1}H NMR Spectrum (DMSO-d_6, d in ppm):

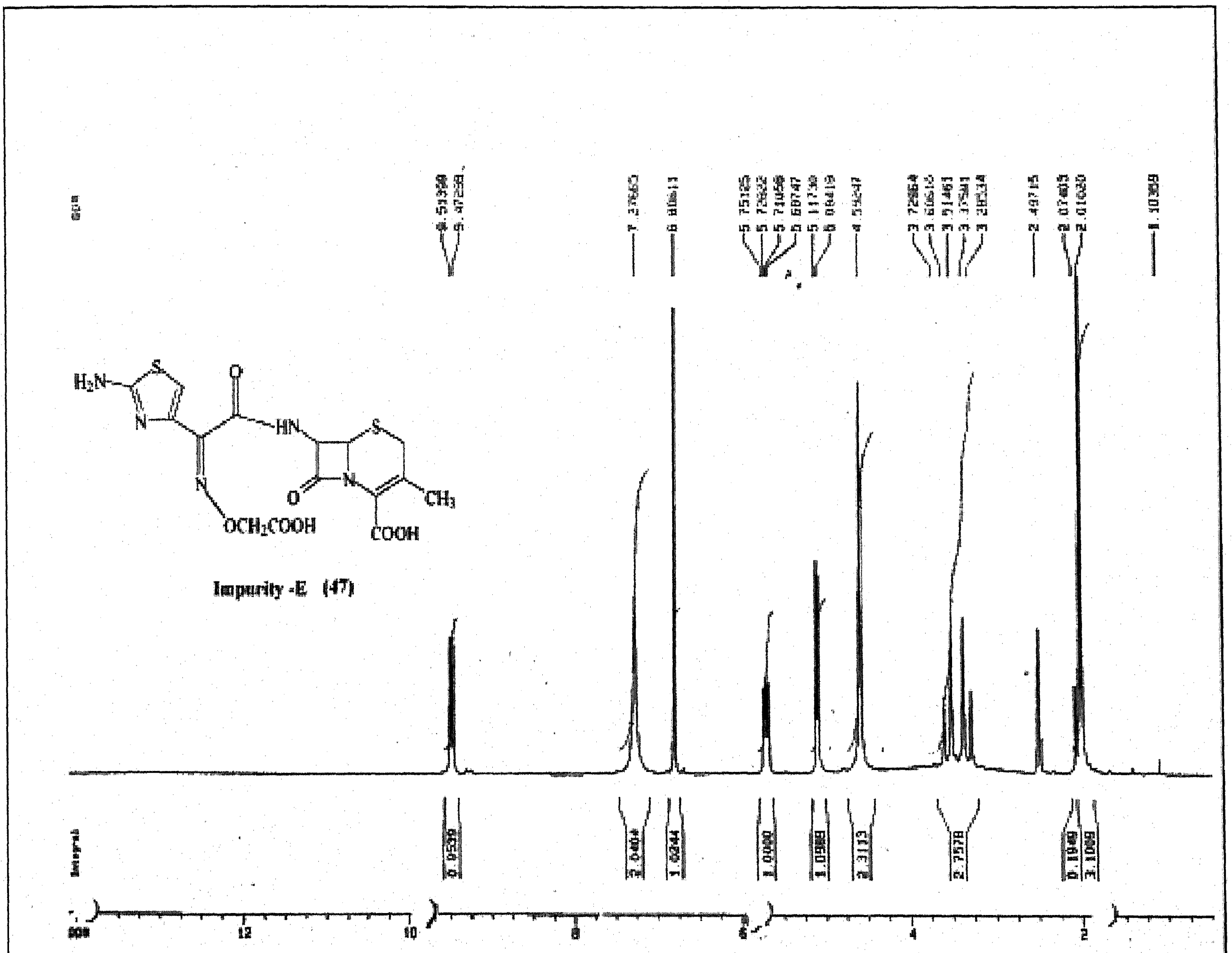
1.40-1.1.48 s (9H, -C $(CH_3)_3$, 3.58-3.3.85 q (2H, -S-CH_2), 4.66 s (2 H, =N-OCH_2COOH), 5.19 d (1H, vinyl-C=CH_2), 5.29 d (1H, vinyl-C=CH_2), 5.53 d (1H, -CO-CH-CH), -5.88 d (1H, - CO-CH-CH), 6.94 s (1H, -S-CH= (amino thiazole)), 7.07-7.21 m (1H, -CH=CH_2), 7.26 s (2H, -NH_2) , 9.6 d (1H, -NH).

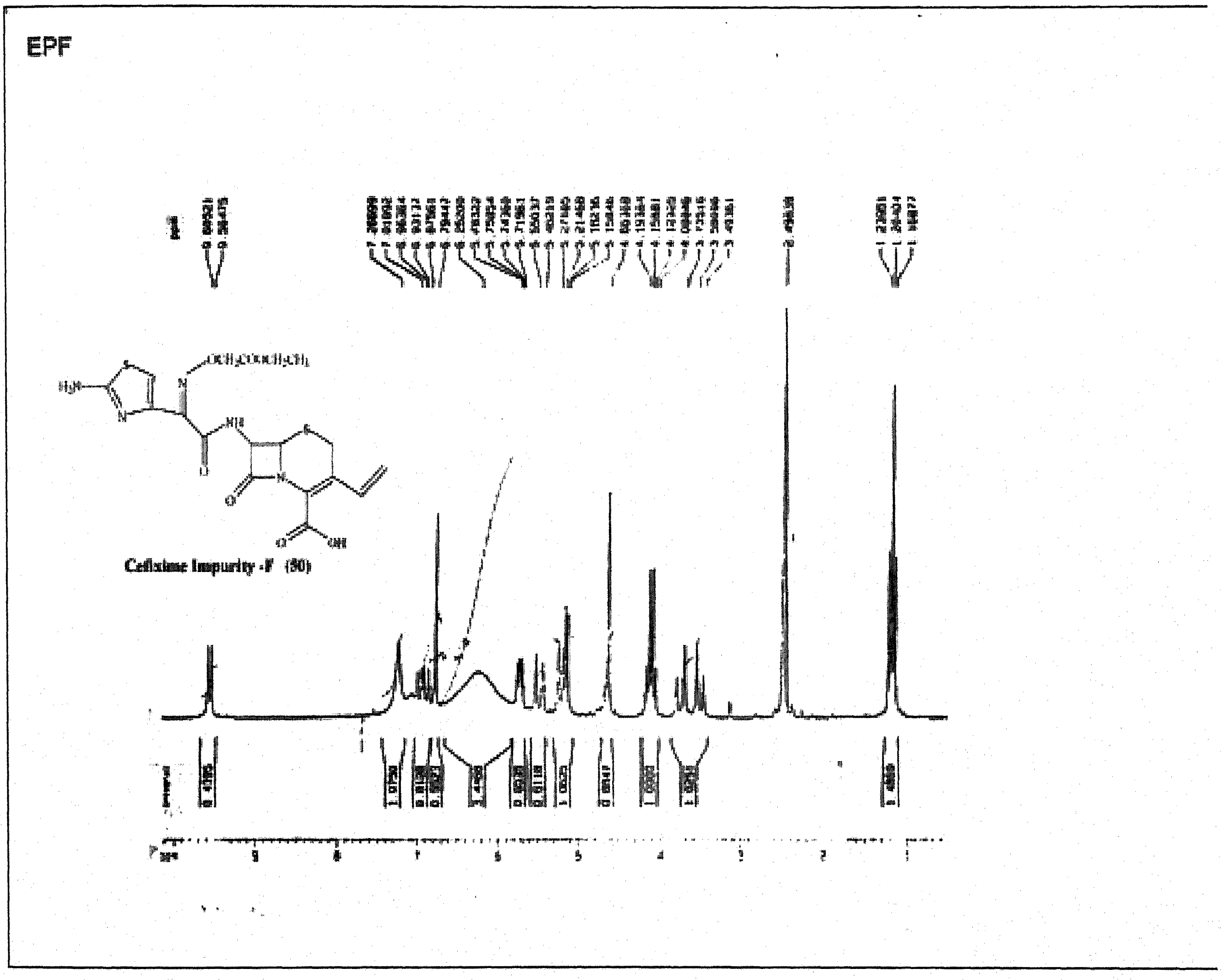

3-VBA
3-VBA (24)


7-AE
7-Amino ester p-TAS salt 26 B

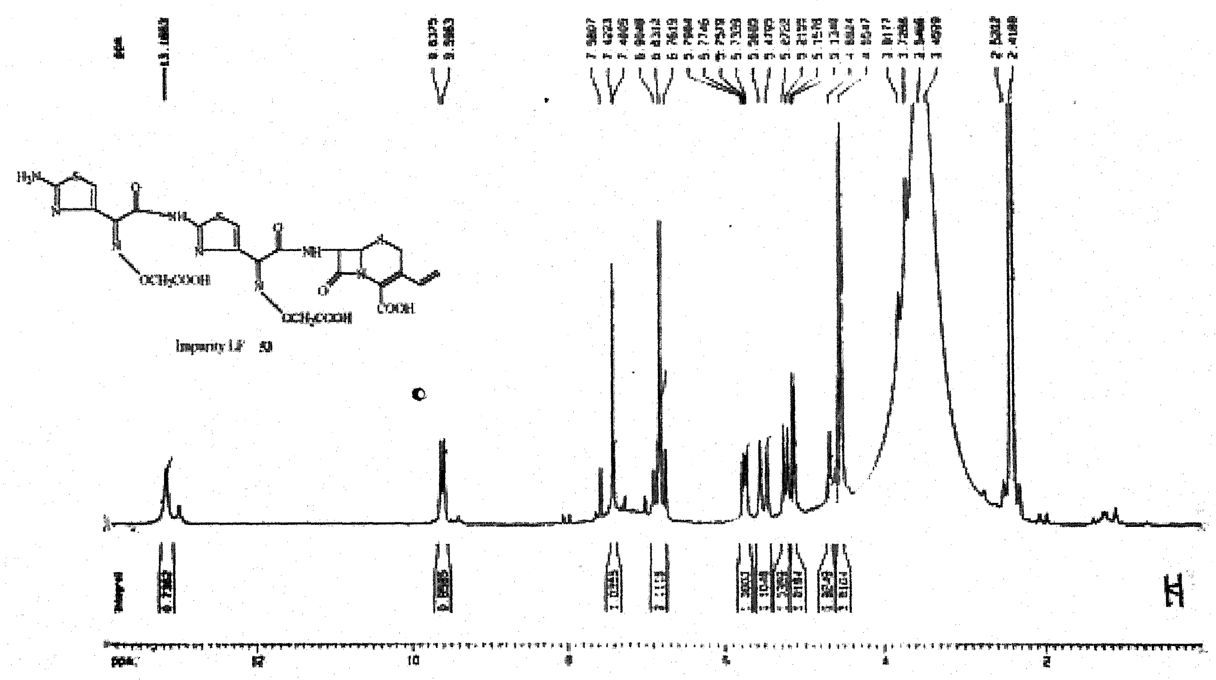


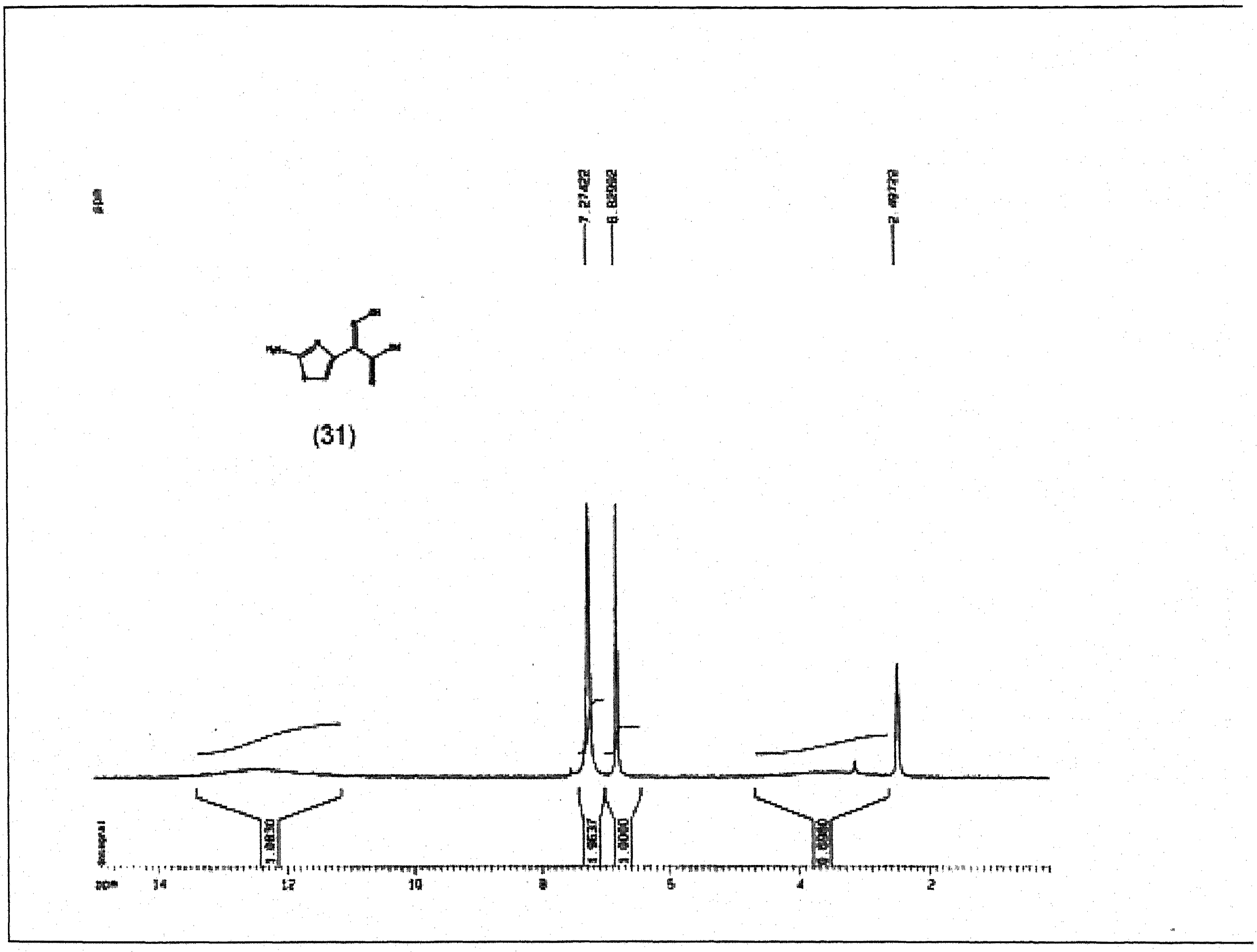

D
Cefixime impurity -D (42)
ppm 10 8 6 4 2 0

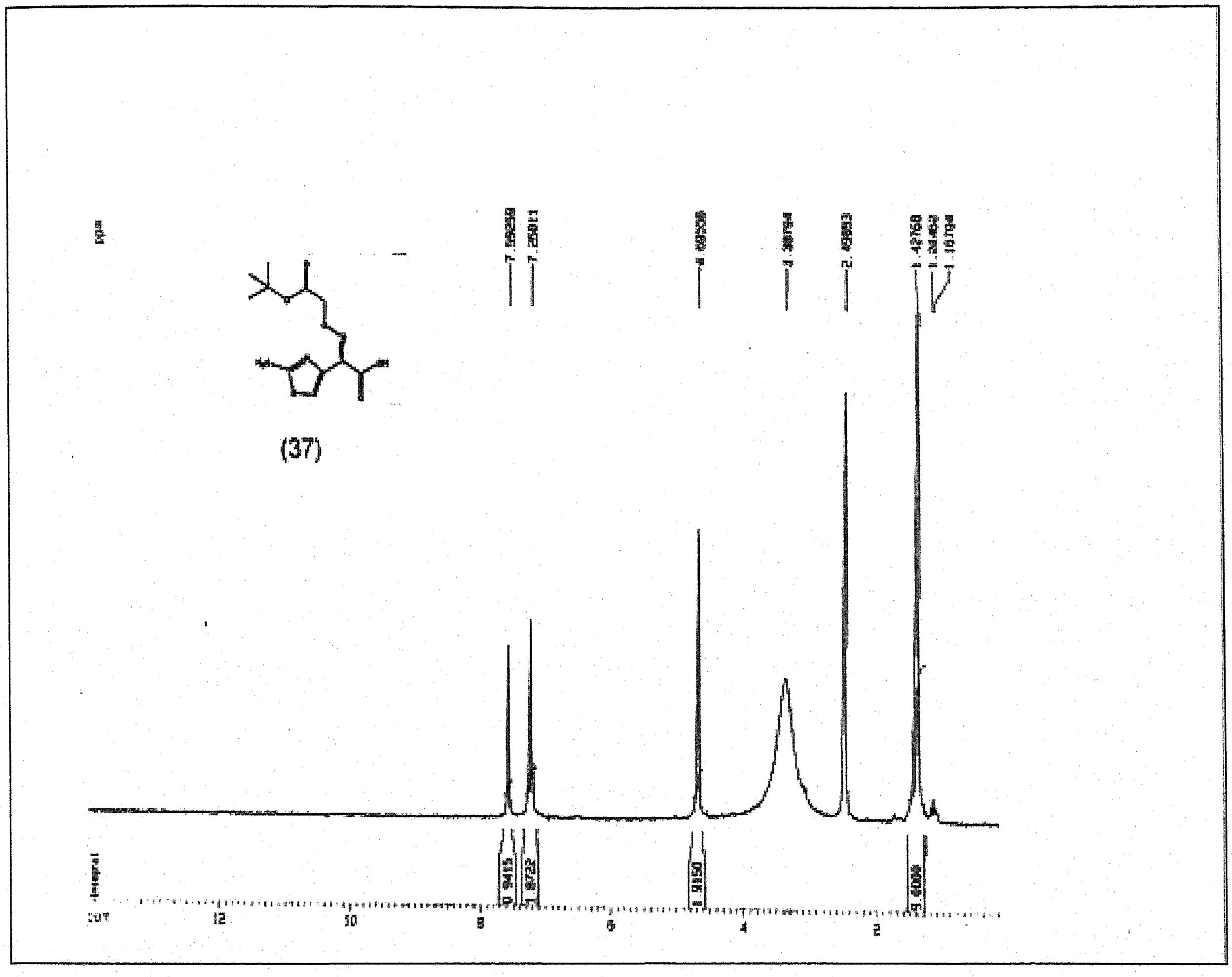

Impurity -E (47)
Integral
ppm


EPF
Cefixime Impurity -F (80)

F







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Chapter -III

Process for manufacture of Cefprozil monohydrate and synthesis of its impurities

3.1. Introduction

Cefprozil monohydrate is a semi-synthetic broad-spectrum Cephalosporin antibiotic consisting of 90:10 *Z/E* isomeric mixture. Cefprozil is an acid-resistant Cephalosporin due to the *para*- hydroxyphenyl-glycyl substituent at the 7 position. It acts by binding with target protein on the cell wall of susceptible bacteria, leading to inhibition of cell wall synthesis & the death of the cell. Its in vitro spectrum of activity is similar to those of known cephalosporins Cefaclor & Cefuroxime axetil, and it is additionally active in vitro against penicillin-resistant strains of Streptococcus pneumoniae and certain anaerobes including Clostridium dificille. Cefprozil monohydrate was discovered and developed by Bristol-Myers.

Cefprozil is a commercially valuable and therapeutically useful oral cephalosporin antibiotic effective in controlling diseases caused by a wide variety of Gram positive and Gram negative microorganisms.

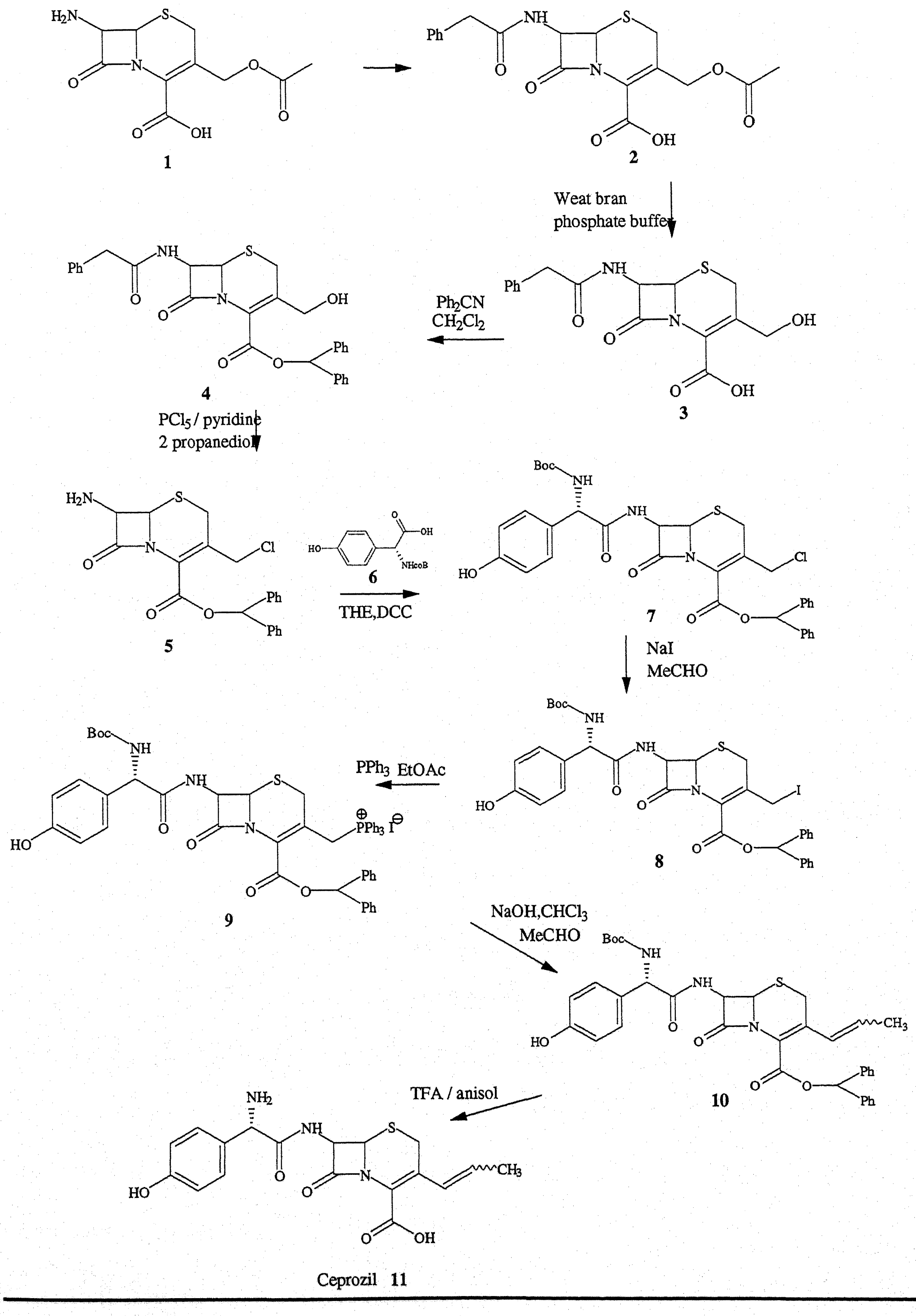
Because of its therapeutic usefulness and efficient broad spectrum of activity, there is always a need for an improved synthetic process which would result in a product with high purity and yield, with minimum level of impurities, preferably absent, coupled with ease of operation and, more importantly, with low production cost.

3.2 Literature methods

In literature methods, synthesis of Cefprozil has essentially been carried out by amidification of a 7-amino-3-(1-propen-1-yl)-cephem derivative with α-amino-p-hydroxyphenylacetic acid or its reactive derivative .

Hideaki Hoshi, et.al[1] reported the synthesis of Cefrpozil monohydrate .It involves use of 7-Amino cephalosporinic acid as starting material, which is converted to benzhydryl-7-amino-3-halomethyl-3-cephem-4-carboxylate. This intermediate on subsequent condensation with D-2-(t-butoxycarbonylamino)-2-(p-hydroxyphenyl) acetic acid in the presence of N,N'-dicyclohexylcarbodiimide (DCC) as the coupling agent followed by Wittig reaction at 3-position. The final deprotection of the carboxy protecting group resulted in the product Cefprozil. The synthetic scheme is illustrated below (**Scheme-3.I**).

Scheme 3.1

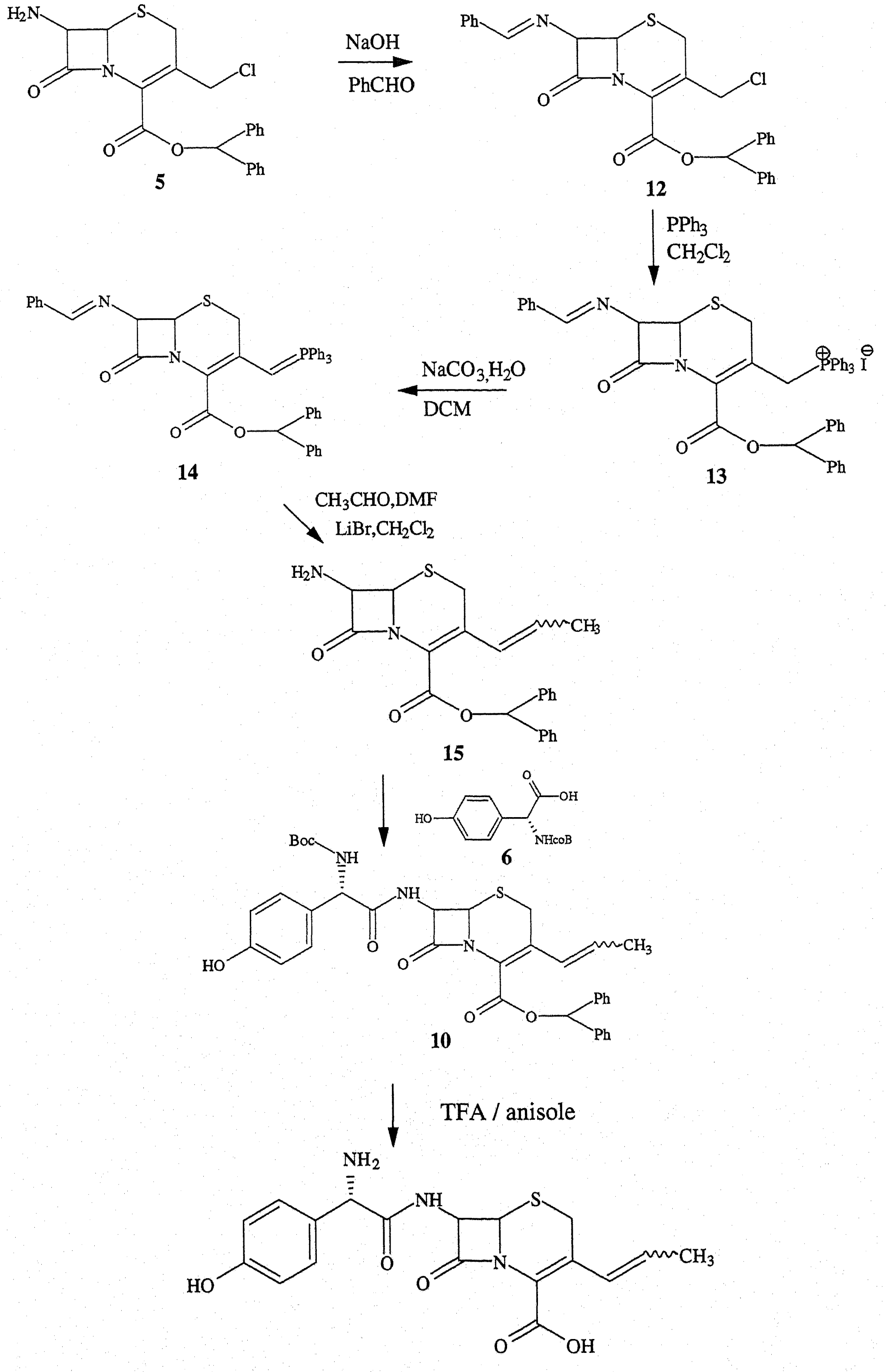

H2N
S
N
O
OH
1
Ph
NH
2
Weat bran
phosphate buffer
3
Ph2CN
CH2Cl2
4
Ph
PCl5 / pyridine
2 propanediol
5
Cl
HO
6
NHcoB
THE,DCC
Boc
7
NaI
MeCHO
I
8
PPh3 EtOAc
PPh3 I
9
NaOH,CHCl3
MeCHO
CH3
10
TFA / anisol
NH2
Ceprozil 11

One of the limitations of the process is that it employs DCC, which is toxic, expensive and requires rigorous anhydrous conditions. Also dicylohexylurea is formed as a byproduct during the process, removal of which calls for several tedious chromatographic purification and isolation steps to be employed to get the product in pure form.The other limitation is to get almost of Z-isomer in greater than 89% which is active pharmaceutical ingredient.

Hideaki Hoshi, Ichikawa, Jun Okumara et.al[2] reported an alternative synthesis of the Cefprozil by introducing propenyl group at C3 position of Cephem compound (**15**) by a Wittig reaction of the triphenylphosphoranyl intermediate derived from a 3-chloromethylcephem compound (**5**) with acetaldehyde using 10 equivalents of lithium halides such as lithium chloride, lithium bromide or lithium iodide to achieve a Z to E ratio of 9:1. The reaction is carried out in dichloromethane and a co-solvent selected form dimethyl formamide or isopropyl alcohol at a temperature 0 to 25°C; yield 71%; reaction time of 20 to 24 hours. The cephem compound (**14**) is further deacylated using phosphorous pentachloride in presence of a organic base such as pyridine in dichloromethane followed by alcoholysis using 2 moles of 1,3-butanediol at –20°C; followed by deprotecting the carboxy group using 20 equivalents of TFA in anisole at 0°C to yielded the intermediate 7-Amino-3-(propen-1-yl)-3-cephem-4-carboxylic acid (7-APCA,**16**) which on condensation with D-p-hydroxyphenylglycyl chloride hydrochloride in presence of organic base affording Cefprozil (**11**) .

This method suffers from a limitation in that it utilizes a large excess of expensive lithium halide such as lithium bromide that is not cost effective for an industrial scale production. The synthetic scheme is illustrated below (Scheme-**3.2**)

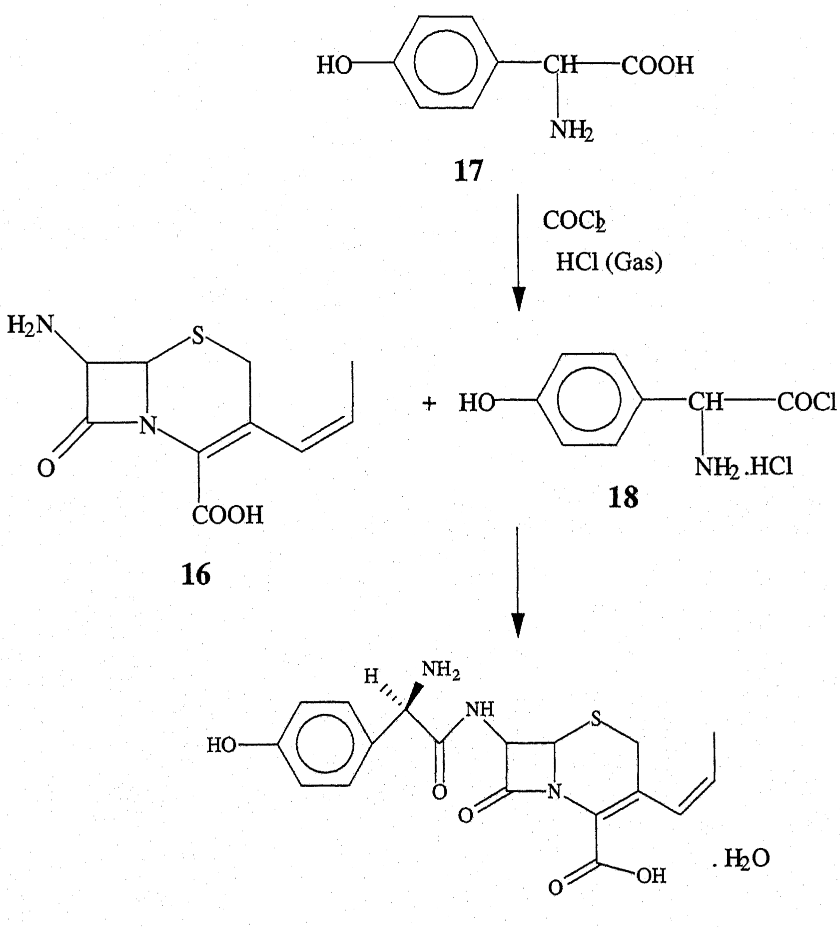
Scheme : 3.2



Cefprozil (11)

Vuaille ; Andre et.al[3] describe methods for preparation of Cefprozil, which generally comprise reaction of 4-hydroxyphenylglycine with phosgene, followed by addition of gaseous hydrogen chloride to give 4-hydroxyphenylglycine chloride hydrochloride(**18**) This is further reacted with a suitable 7-amino-3-propenyl-3-cephem –4-carboxylic acid (**16**) to give the desired Cefprozil monohydrate . The synthetic scheme is illustrated below (Scheme-**3.3**).

Scheme - 3.3

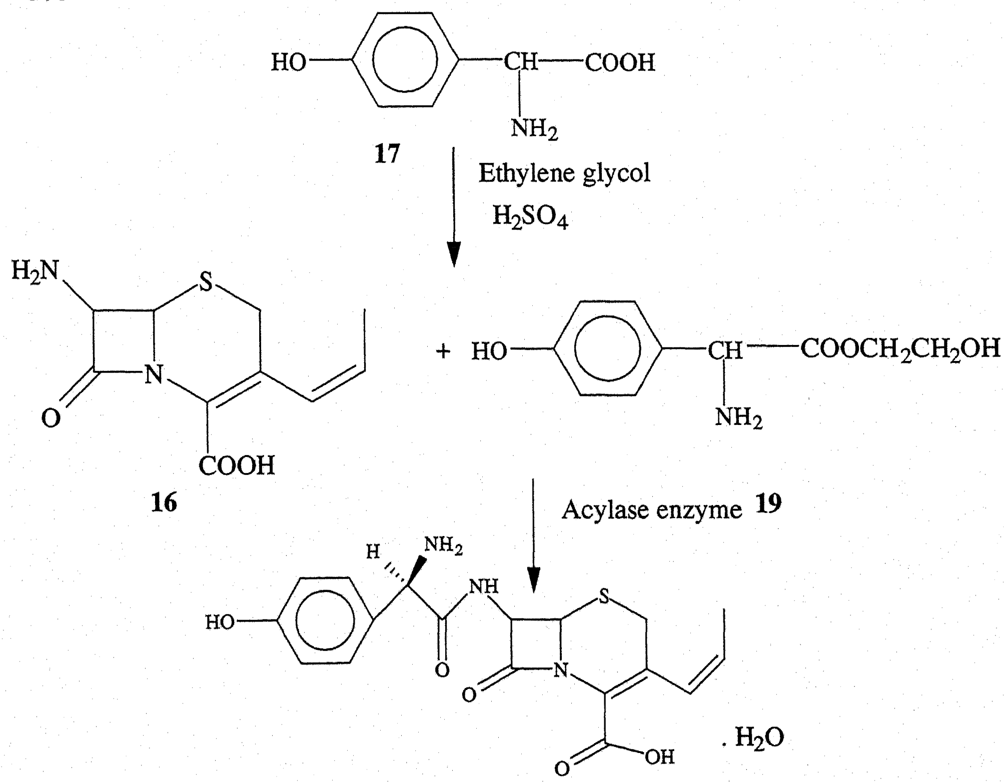


Cefprozil monohydrate (11)

However, these methods employ toxic and hazardous phosgene and gaseous Hydrochloride, which are difficult to handle on an industrial scale and cause environmental problem.

Usher.John. et.al[4] reported the method for preparation of Cefprozil comprising reaction of 4-hydroxyphenylglycine (**17**) with ethylene glycol to give an ester which is reacted with 7-Amino-3-(propen-1-yl)-3-cephem-4-carboxylic acid (7-APCA, **16**), in presence of enzyme, acylase. However, this method utilizes excess amount of the expensive enzyme rendering the method uneconomical. The synthetic scheme is illustrated below (Scheme-**3.4**)

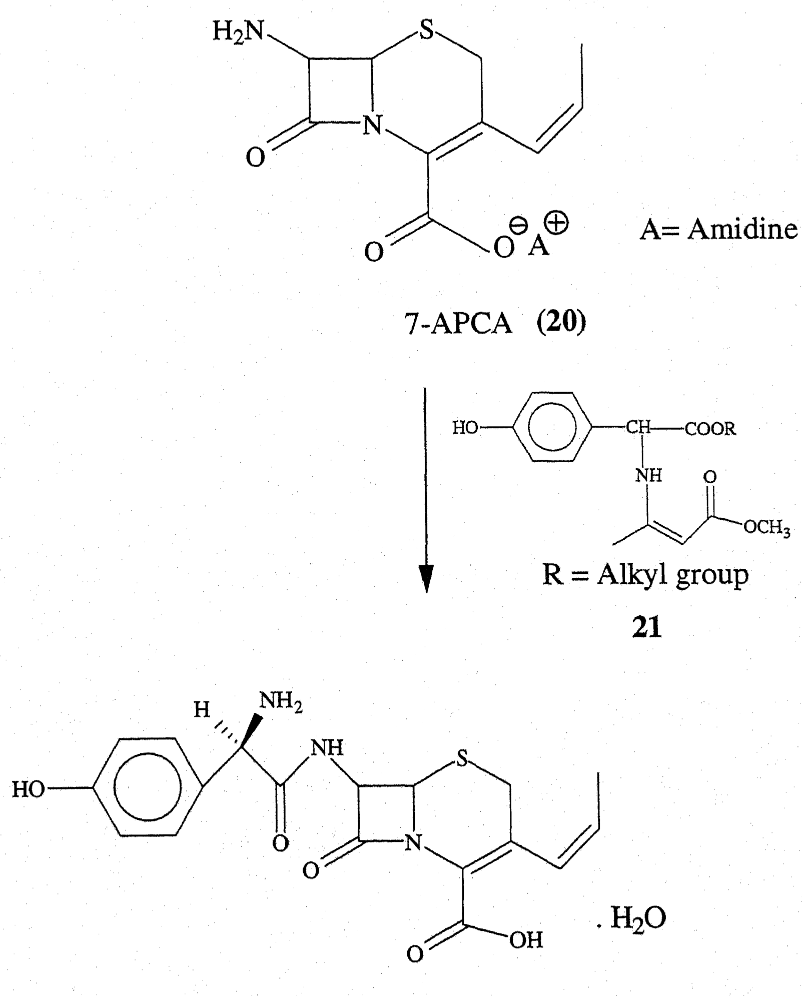
Scheme - 3.4



Cefprozil monohydrate **(11)**

Greil, Ludescher J. et al.[5] given the synthesis of Cefprozil from salt of 7-APCA with amidine and its use in the production The application describes the synthesis of Cefprozil by the reaction of an amidine salt of 7-APCA (**20**)a mixed carboxylic acid anhydride of a N-substituted-α–amino-p-hydroxyphenylacetic acid (**21**). The patent does not comment on the purity or yield of the product. The synthetic scheme is illustrated below (Scheme-**3.5**).

Scheme -3.5



Cefprozil **(11)**

All the above-described methods discussed herein are associated with the formation of varying amounts of impurities, which affect the overall yield and the quality of the product. Also removal of these impurities calls for additional purification and isolation steps, which render the process lengthy and tedious

Cephalosporin antibiotics carrying the D-α-amino-α-(4-hydroxyphenyl) acetamido addendum at the 7-position such as Cefprozil and Cefadroxil are generally prepared by reacting the respective 7-amino-3-substituted-3-cephem-4-carboxylic acid or its salt/derivative with an activated derivative of 4-hydroxyphenylglycine such as a reactive ester, a reactive amide or a mixed acid anhydride. However, use of reactive amide or esters makes it difficult to obtain the desired product in high purity and yield because of the occurrence of side-reactions as well as racemization.[5]

. Hideaki Hoshi, et.al[2] described method of deacylation using phosphorous pentachloride in presence of a organic base such as pyridine in dichloromethane[7] followed by alcoholysis using 2 moles of 1,3-butanediol at –20°C; deprotecting the carboxy group using 20 equivalents of TFA in anisole at 0°C This method suffers from a limitation in that it utilizes a large excess of expensive trifluoroacetic acid for de-esterification that is not cost effective for an industrial scale production.

Kameyama; et.al.[6] were reported method of deprotecting the amino and carboxy by first treating 1 mole of the compound with 1 to 10 moles of phosphorous pentachloride and 1 to 10 moles of a organic base such as pyridine in a chlorinated

hydrocarbon solvent such as methylene chloride used in an amount 1 to 50 liter per kg of the compound at –30 to 30°C, to produce a imino-(-lactam compound which is converted to compound (**16**) by treating with a phenol selected from phenol, cresol, chlorophenol, methoxyphenol or naphthol used in an amount of 0.5 to 200 kg per kg of the compound in presence of a lower aliphatic alcohol such as methanol used in an amount of 0.01 to 0.05 kg per kilogram of the phenol used at a temperature between 0 to 50°C.

The method given by Kameyama; Yutaka, Yamada et.al.[6] suffers in that it uses large excess of phosphorous halide, organic base and solvent leading to a large reaction mass.

Lanz; et.al[8] have described a method for preparing compound of formula (**16**) from a carboxy ester of a alkoxycarbonyl or a aryloxycarbonyl protected amino compound of formula (**15**) in a single step process comprising of treating with a strong acid such as formic acid, trifluoro acetic acid, alkyl or arylsulphonic acid or Lewis acids like Aluminum halides, boron halides, silyl halides in a solvent such as anisole or diethyl ether. The yields of the process vary from 19 to 72%.,

The method uses large excess of trifluoroacetic acid for deprotection of amino and carboxyl groups.

There have been reported many methods for adjusting the Z- to E-isomer ratio in the preparation of 7-amino-3-propenyl-3-cephem-4-carboxylic acid, 7-APCA or

PACA,(**16**) which is the key intermediate in the synthesis of 3-propenyl Cephalosporin antibiotics such as Cefprozil(**11**). There have been reported methods to obtain (Z)-enriched Wittig product by using Lithium salts and suitable solvents as mentioned above. Also, there are several reported methods to enrich the (Z)-isomer content of (E) and (Z) mixture of 7-APCA(**16**) by derivatisation and crystallization, exploiting solubility differences of various salts of the E- and Z-isomers, and chromatographic separations. For example,

Murray A. Kaplan, et.al[9] described a process for preparing Cefprozil that is substantially free from the corresponding E-isomer. The process involves preparation of the sodium salt of imidazolidinone derivative of a mixture containing Cefprozil and its corresponding E-isomer, and separation of the imidazolidinone derivative isomers based on their differential solubility.

Ludescher, et.al[10] have disclosed a process for preparing (Z)-isomer enriched 7-amino-3-propenyl-3-cephem-4-carboxylic acid (7-APCA, **16**) depleting the corresponding (E)-isomer in a mixture of the (Z)- and (E)-isomers of 7-APCA by subjecting a solution of the mixture to adsorption chromatography.

Ludescher, et.al[11] provided a process for preparing a (Z)-isomer enriched 7-APCA(**16**) by reacting a mixture of (Z) and (E) isomers with a lithium, sodium or potassium base, ammonia or amine to form a mixture of the (Z)- and (E)-isomer of the corresponding slats and depleting the (E)-isomer salt from (Z)-isomer salt in a

solvent mixture in which the two isomers have different solubility to recover the enriched (Z)-isomer salt of 7-APCA(**16**) and converting it to the free acid.

Kumar Yatendra, et.al[12] have reported a method of preparing (Z)-isomer enriched 7-APCA by reacting the mixture of (Z)- and (E)-isomers of 7-APCA with a ketone in the presence of an inorganic acid such as HCl, HBr, HI, H_2SO_4 and $HClO_4$ to form a alkylidene ammonio salt derivative of 7-APCA, obtaining the (Z)-isomer enriched alkylidene ammonio salt derivative of 7-APCA by crystallizing at a temperature between 0 to 30°C. The 7-APCA (**16**) was regenerated from the alkylideneammonio salt derivative by suspending in water at a pH of 8.0-8.5 to obtain a clear solution and then treating with activated charcoal and acidifying with 6N HCl.

3.3 Objective and strategy

The object of the present work is to synthesize Cefprozil in high purity, substantially free of impurities by a simple and cost-effective industrially feasible method, which comprises preparation of mixed acid anhydride and its condensation with a protected 7-APCA (**16**).

It is also an object of the present work to provide an improved method of preparation of mixed acid anhydride by selecting the sequence and temperature of addition of the reagents, which will result in minimization of impurities.

The need of simple and cost-effective method for the preparation of Cefprozil in high purity and yield could be met through minimization of the impurities associated

with the reported methods with concurrent improvement in the purity and yield of the product. The steps involved in the reported synthesis of Cefprozil (**11**) are minimized and the manufacturing cost was reduced by improving yield and reduction of reactant used.

3.4 Present work

3.4.1 Selection of synthetic scheme

During the course of the present work, we have reproduced the process for preparation of Cefprozil as described by Greil, Ludescher J. et al[5].. It was found that the preparation of mixed acid anhydride by the method reported by,Greil, Ludescher J. et al. and its subsequent reaction with amidine salt of 7-APCA is associated with the formation of impurities in the range of 6-7%.

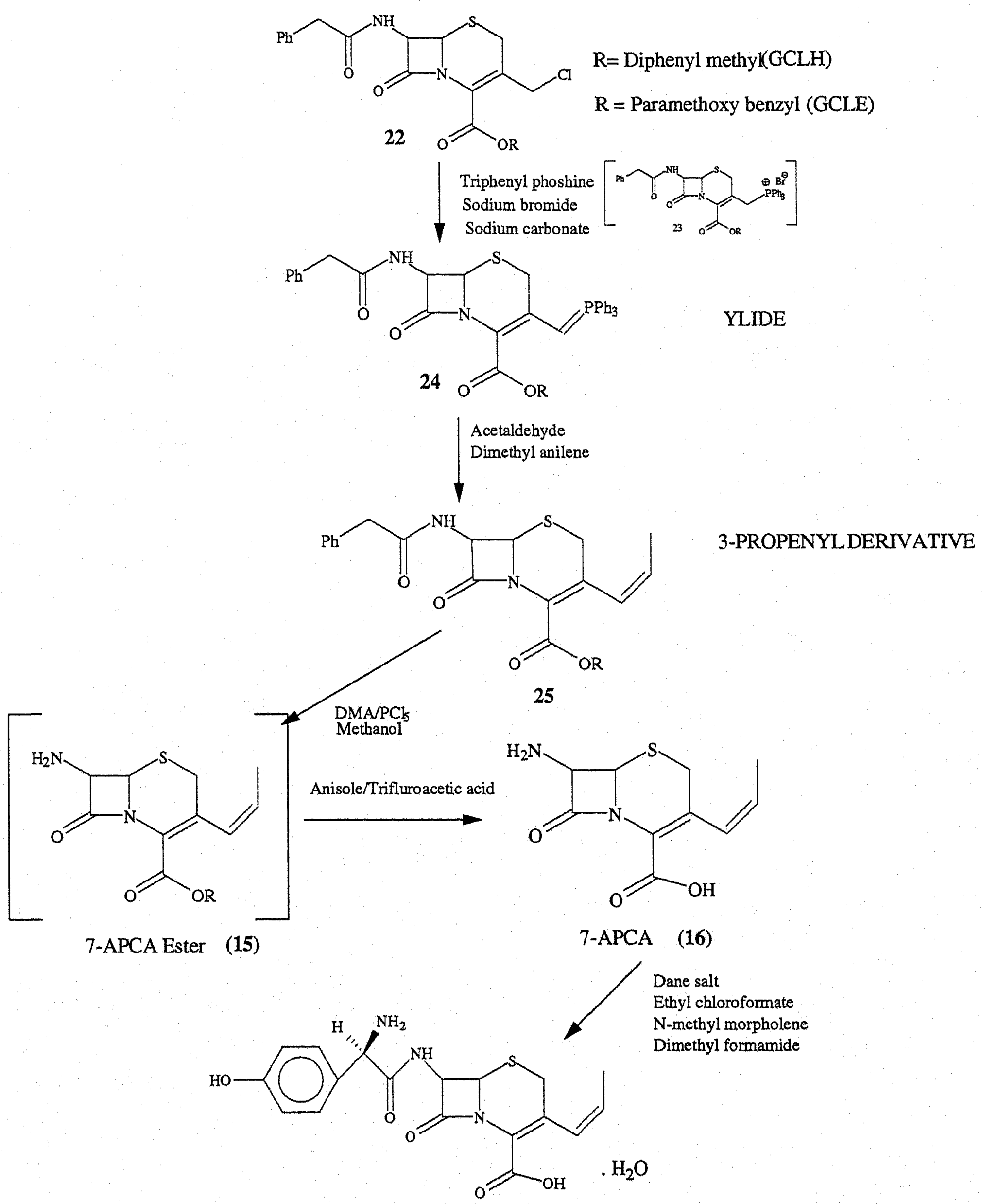
In the literature methods, Cephalosporin antibiotic such as Cefprozil have been prepared by reacting the mixed acid anhydride with respective 7-amino-3-substituted-3-cephem-4-carboxylic acid or its salt/ derivative such as an amidine salt of 7-APCA as reported by Greil, Ludescher J. et al[5]. However, use of 7-amino-3-substituted-3-cephem-4-carboxylic acid, its acid salt or an amidine salt reported in these above described methods is found to give the product in low yield due to side reactions of the unprotected 4-carboxylic acid group and 7-amino group.

Hence there is a need to for a protected form of 7-APCA, which will activate the amino group in the 7-position, efficiently protect the carboxylic acid group, which will

not require additional deprotection steps and can be deprotected, in-situ during reaction work-up.

Based on drawbacks of these reported methods for the synthesis of the Cefprozil we proposed the efficient and cost effective industrially viable process, which is mentioned in scheme (**3.6**)

Scheme : 3.6



Cefprozil (11)

3.4.2 Preparation of 4-methoxybenzyl 7-phenylacetamido-3-[(Z/E)-propenyl]-3-cephem-4-carboxylate.

The preparation of 3-propenyl Cephalosporin derivatives can be proceeds through a reaction sequence involving the intermediacy of phosphonium salt and phosphoranylidene intermediates that are fast degrading. By-products are often formed due to decomposition of the intermediates during isolation. Thus, it is difficult to obtain a Wittig product of high purity in good yield. Further, the Wittig reaction generally yield mixtures containing both the (Z)- and (E)-isomers. The Z-configuration of the 3-propenyl groups is related to the activity of 3-propenyl Cephalosporin antibiotics against gram-negative bacteria, hence, there is a need to minimize the undesired (E)-isomer in these antibiotics. For example, Cefprozil has a limit of Z-isomer content in the range of 89 to 94%.

In our objective, we found that, Wittig reaction to introduce the alkenyl group at C3 position can be effectively carried out using less expensive sodium bromide in presence or absence of a catalytic amount of a hydrohalic acid. Carrying out the Wittig reaction in one-pot synthesis improved the yield and purity of the Wittig product.

Reaction of GCLE (**22**), in presence of sodium halide and triphenyl phosphine in the mixture of Dimethyl formamide and methylene chloride yielded the corresponding phosphonium salts which on reaction with acetaldehyde in presence of sodium hydroxide furnished 4-p-methoxybenzyl-3-propenyl-7-phenyl-acetamidocephem carboxylate (3-PGCLE, **25**).

For preparing the phosphonium salt (**23**), different metal halides such as sodium iodide, sodium bromide, and Lithium bromide were experimented in various solvents such as acetone/ isopropyl alcohol/and mixture of DMF and dichloromethane. Based on the cost consideration and quantity required for the reaction, sodium bromide was the metal halide of choice for preparing the phosphonium salts.

Phosphonium salt (**23**), formation was found to be facile in polar solvent, particularly in DMF with sodium bromide. Other polar solvents such as isopropyl alcohol did not result in the completion of reaction.

With the view to combine, phosphonium salt (**23**), formation step & the subsequent step (Wittig reaction), the phosphonium salt formation in the mixture of dichloromethane & DMF was selected. Considering the rate and ease of reaction 2:1 ratio of DMF / DCM was fixed for carrying out the reaction.

For converting the phosphonium salt to ylide (**24**), number of bases such as sodium carbonate sodium hydroxide & sodium bicarbonate have been tried. Since sodium hydroxide gave comparatively better results, sodium hydroxide was gives results in Wittig reaction.

Effect of temperature on the Wittig reaction was studied at various the temperatures from 5-30°C. There was no effect on the yield and quality up to 5-10°C whereas higher temperature (25-30°C) led to the poor yield & more impurity formation.

In the conversion of ylide (**24**) to 4-p-methoxybenzyl-3-propenyl-7-phenyl-acetamidocephem carboxylate (3-PGCLE, **25**), 20-30% aqueous acetaldehyde solution as well as neat acetaldehyde (95-98%) was tried. Reaction conversion was found better by using neat acetaldehyde, which was enhanced the yield and quality of the product.

Sodium chloride solution (20%) was played important in the Wittig reaction which helps to control the impurity formation and to improve the yield and quality of the product .

For isolating the final product in pure form, dichloromethane was removed at atmospheric pressure and isolation of 3-PGCLE (**25)** was studied using solvents like methanol, ethanol and isopropyl alcohol. Isopropyl alcohol was found to be a solvent of choice, since use of ethanol and methanol gave a poor quality of product.

The process, which emerged after studying all these parameters, is given as follows ,

Reacting the compound (22) with triphenyl phosphine in the presence N,N-Dimethyl formamide , sodium bromide and optionally in presence of catalytic amount of a hydrobromic acid to get the intermediate triphenyl phosphonium salt (**23**) and reacting the triphenyl phosphonium salt with a sodium hydroxide as a base to get the intermediate (**24**),

Reacting the intermediate (**24**) with a acetaldehyde (~98%), in the presence of catalytic sodium hydroxide as base at a temperature in the range of 5 to 15°C, to produce a compound of formula **(25)** with required ratio of Z and E isomer

3.4.3 Preparation of 7-amino-3-[(Z/E)-propenyl]-3-cephem-4-carboxylic acid

p-Methoxybenzyl-7-amino-3-Propenyl cephem-4-carboxylate, namely 7-APCA ester(**15**), was prepared by the reaction of p-methoxybenzyl-7-phenylacetamido-3-propenyl cephem-4-carboxylate(**25**) with base and PCl_5 to give iminochloride which on treatment with methanol resulted the formation of p-methoxybenzyl-7-amino-3-propeny cephem-4-carboxylate (**15**). This, on *in situ* treatment with trifluoroacetic acid followed by precipitation with sodium hydroxide in D.M. Water resulted in the formation of 7- Amino 3-propenyl Cephalosporinic acid (**16**) .

As per our previous experience in Cefixime molecule deacylation of p-methoxybenzyl-7-phenyl-acetamido-3-propenyl cephem-4-carboxylate, combination of DMA/PCl_5 gave the best results

Since the p-methoxybenzyl-7-amino-3-propenylcephem carboxylate was not isolated, it was directly proceeded to deprotection by in-situ way. Deprotection of 7-APCA ester (**15**) was tried with different reagents such as TFA / Anisole[14], TFA / DCM[13], Aluminum chloride anisole[15], phenol[13] among these all Trifluoroacetic acid / Dichloromethane combination was found to be suitable for the deprotection in terms of low cost of the process.

Deprotection of 7-APCA ester (15) was attempted at different temperature from 5 to 30°C. The rate of reaction is slow at lower temperature [(5-10°C) reaction require ~20 hrs for completion whereas at higher temperature (25-30°C) impurity formation

was more. Better results were obtained at 18-20°C in terms of a yield and quality of the intermediate.

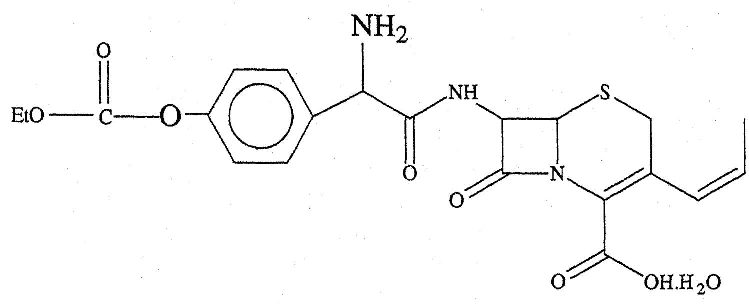
The detailed process that emerged from the optimization of the above parameters is given in experimental section.

The deacylation and de-esterification can be sequentially achieved in one pot by in-situ method. Deacylating the compound of formula (**25**) using a phosphorous pentachloride, in presence of a Dimethyl aniline and catalytic amount of chlorotrimethyl silane in the presence of dichloromethane as a solvent, followed by alcoholysis using a methyl alcohol at temperature range of –50 to--20°C to yield a compound of formula (**15**), which on de-esterified using TFA at 20±2°C to get a compound of formula (**16**).

All these improvements in conjunction have the advantage in providing compound of formula (**11**) of high quality and in high yields, which moreover is convenient and cost effective.

3.4.4 Preparation of 7-[D-α-amino-α-(4-hydroxyphenyl) acetamido]-3-(1-propen-1-yl)-3-cephem-4-carboxylic acid (Cefprozil monohydrate ,11)

In the literature reported methods, wherein the sequence of addition is such that the Dane salt and the acylating agent are added first, the free acylating agent tends to react with the hydroxy group and leads to the formation of impurity (**26**) in higher quantities.



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The method for the preparation of mixed anhydride as described by Greil, Ludescher J. et al[5]. comprises addition of 4-picoline and Dane salt to a mixture of DCM and DMF at ambient temperature and cooling the suspension to –30°C followed by addition of the acylating agent and agitating the suspension at –25° to –20°C and cooling it to –50°C, followed by its reaction with an amidine salt of 7-APCA(**20**), then 6-7% impurity is observed in the reaction mass as against the 2.26% impurity observed in the method of present invention.

Hence there is a need to have protected form of 7-APCA, which will activate the amino group in the 7-position, efficiently protect the carboxylic acid group, which will not require additional deprotection steps and can be deprotected in-situ during reaction work-up.

As per the process described by Barnish; et. Al[16] the mixed anhydride is prepared by adding a chloroformate, such as ethylchloroformate, to a solution of N-protected-4-hydroxy phenylglycine dissolved in an inert organic solvent at a temperature of –5° to 0°C in the presence of a base.

Most of the literature described the methods for preparation of the mixed acid anhydride are associated with the formation of varying amounts of different impurities. For example, during the preparation of mixed the 4-hydroxy group of the Dane salt is likely to react with the acylating agent thereby forming an impurity which further reacts with 7-APCA or its salts to form an impurity (**26**).

Limitation of the reported methods for preparation of Cefprozil:

i) Utilize toxic and expensive chemicals such as phosgene, DCC and HCl;

ii) Utilize expensive enzyme like acylase; and

iii) Are associated with formation of varying amounts of impurities which give the product in low purity and yield, rendering such methods less cost effective.

Therefore, a need exists for a simple and cost-effective method for the preparation of Cefprozil in high purity and yield. Such a need could be met through minimization of the impurities associated with the reported methods with concurrent improvement in the purity and yield of the product.

In the present work we have found that the mixed carboxylic acid anhydride of a N-substituted-α-amino-p-hydroxy phenyl acetic acid or its salt (Dane salt,**21**) can be prepared by a careful selection of a specific sequence and temperature for addition of the reagents so that it will result in minimization of impurities during product formation.

The effect of varying sequence of addition of reagents during mixed anhydride preparation on the amount of total impurities formed along with Cefprozil was established by the following experimental evidence.

Cefprozil monohydrate is prepared by silylating the 7-APCA with the mixture of hexamethyl disilazane and trimethylchlorosilane in presence of N,O-Bis(trimethylsilyl)acetamide to get silylated 7-APCA, which on condensed with mixed anhydride of p-Hydroxy phenyl glycine dane salt (potassium methyl) and ethylchloroformate.The condensed product was hydrolyzed with dilute hydrochloric acid and subsequent treatment in a mixture of Acetone-Dimethyl formamide to obtain Cefprozil solvate, which on desolvation with the mixture of DM Water and ethyl acetate to furnish the Cefprozil monohydrate.

3.4.4.1 Role of silylating reagent

Silylation of 7-APCA was tried by using the mixture of hexamethyldisilazane(HMDS) and trimethylchlorosilane(TMCS) with various molar ratio such as hexamethyl disilazane (0.65-1.0 mole) and trimethyl chlorosilane (0.03–0.82 mole). All combination of reagents almost gave similar results, hexamethyl disilazane (0.80 mole) and trimethyl chlorosilane (0.82 mole) is choice of the combination. Results of the various experiments are listed in Table –3.

3.4.4. 2 Effect of catalyst on Silylation of 7- APCA

Different catalyst were attempted in Silylation of 7- APCA such as p-Toluene sulphonamide, acetamide, imidazole, N,O-Bis(trimethylsilyl)acetamide (BSA). Al

catalyst were gives the similar results. N,O-Bis(trimethylsilyl)acetamide (BSA) is the choice of the catalyst as it is easy to handle at small quantity (Table–4).

Table–3

S No	HMDS (moles)	TMCS (moles)	Unreacted % 7-APCA in reaction
1	1.20	0.03	1.2
2	0.95	0.80	3.77
3	0.85	0.80	1.15
4	0.80	0.65	2.2
5	O.80	0.82	0.5
6	0.70	0.72	4.1

Table–4

S.No.	Catalyst	Unreacted % 7-APCA in reaction
1	p-Toluene sulphonamide,	1.15
2	Acetamide,	2.11
3	Imidazole	1.2
4	N, O-Bis (trimethylsilyl) acetamide	1.0

3.4.4.3 Effect of various reagents on the preparation of mixed anhydride Mixed anhydride of p-Hydroxy phenyl glycine dane salt (potassium, methyl) was prepared by ethylchloroformate in the mixture of dichloromethane-dimethyl formamide and N-Methyl morpholine as a catalyst.

3.4.4.4 Role of Dimethyl formamide

The quantity of dimethyl formamide varied from (2 to 4.5 V /g 7-APCA) in the preparation of mixed anhydride. Dimethyl formamide (3.5 V) was found to be suitable for the reaction (Table–5a)

Mode of addition of dimethyl formamide plays important role in the preparation of mixed anhydride to control the carbonate impurity. Addition of dimethyl formamide before (temp 20-25°C) Dane salt reduces the carbonate impurity where as addition of dimethyl formamide after (–50 to –55°C) Dane salt enhance the carbonate impurity (Table-5b)

Table-5a

S.No.	**Dimethyl formamide** Volume/gm 7-APCA	Unreacted % 7-APCA in reaction
1	2.0	1.2
2	2.5	3.9
3	3.0	1.15
4	3.50	0.5
5	4.0	3.80
6	4.5	0.7

Table-5b

S.No.	Mode of DMF addition	% Carbonate impurity
1	Before Dane salt	0.10
2	After Dane salt	0.41

3.4.4.5 Effect of base catalyst

Different base catalyst were tried in the preparation of mixed anhydride like Di-sodium hydrogen ortho phosphate, N, N-Dimethyl amino pyridine, pyridine and N-methyl morpholine. Out of these N- methyl morpholine is a suitable catalyst for the completion of reaction and it is optimized to 1% (Table–6

Table-6 :

S.No.	Base catalyst in % w.r.t. 7-APCA	Unreacted %7-APCA in reaction
1	Di-sodium hydrogen ortho phosphate (20%)	19
2	N,N-Dimethyl amino pyridine (1.2%)	40
3	Pyridine (2.0%)	9.88
4	N- methyl morpholine (0.6%)	3.8
5	N- methyl morpholine (0.8%)	0.8
6	N- methyl morpholine (1.0%)	0.25
7	N- methyl morpholine (2.1%)	2.2
8	N- methyl morpholine (2.5%)	1.15
9	N- methyl morpholine (5.0%)	41.0

3.4.4.6 Effect of ethyl chloroformate

Ethyl chloroformate in different molar ratio were attempted from 1.10 to 1.18 mole, better conversion of the reaction obtained in 1.12 mole. Results are shown as below (Table-7)

Table-7

Quantity of ethyl chloroformate in mole w.r.t. 7-APCA	Unreacted % 7-APCA in reaction
1.10	3.91
1.12	1.7
1.15	3.77
1.18	1.15

In the present work, the preferred sequence of addition is such that the acylating agent and base are mixed first so that they form a complex. Dane salt is then added so that the acylating agent-base complex reacts preferentially with the carboxylic acid group and very little amount of the free acylating agent is available for reaction with the hydroxyl group and hence results in reduced quantities of impurity (**26**) as well as other impurities.

(i) If the sequence of addition of the reagents during mixed anhydride preparation is altered in such a way that the Dane salt was first added to a mixture of dimethyl formamide and dichloromethane at –50, the temperature of the suspension was raised to ambient followed by addition of ethyl

chloroformate and N-methyl morpholine, then the product Cefprozil is found to contain total impurities to the tune of 4.6%.

(ii) If the Dane salt is first added to a mixture of dimethyl formamide and dichloromethane at –50 °C, the temperature of the suspension is raised to ambient followed by addition of N-methyl morpholine and then ethyl chloroformate, total impurities observed, after condensation with disilylated 7-APCA, amount to 2.94%.

(iii) If the sequence of addition was such that the ethyl chloroformate and N-methyl morpholine was added to a mixture of dichloromethane and dimethyl formamide at ambient temperature, the suspension was cooled to –35° to –50°C followed by addition of Dane salt, and the mixed anhydride thus prepared is reacted with a silylated derivative of 7-APCA the total impurities are reduced to 2.26 %.

iv) Further, if the ethyl chloroformate and N-methyl morpholine were added to dichloromethane at ambient temperature, the suspension is cooled to –35° to –50°C, Dane salt is added to the cooled suspension, followed by addition of a of a dimethyl formamide to the solution, then the total impurities formed along with Cefprozil are reduced to 0.64 %. The qualitative results as monitored after condensation reaction by HPLC are tabulated herein below.

Effect of sequence of addition of reagents in preparation of mixed anhydride on the level of impurities.

HPLC monitoring method results Table -7

Sequence of addition of ethyl chloroformate during mixed anhydride preparation.	Unconverted starting material %	Product %	Carbonate impurity (26) formed during reaction %	Total impurity formed during reaction, %
(i)Dichloromethane, dimethyl formamide, Dane salt, ethy chloroformate and N-methyl morpholine	5.10	90.3	2.31	4.6
(ii) Dichloromethane, dimethyl formamide, Dane salt, N-methyl morpholine and ethyl chloroformate	2.97	94.09	1.81	2.94
(iii) Dichloromethane, dimethyl formamide, ethyl chloroformate, N-methyl morpholine and Dane salt	4.99	92.74	0.3	2.26

(iv) Dichloromethane, , ethyl chloroformate, N-methyl morpholine , Dane salt and dimethyl formamide	0.56	98.78	0.45	0.64

3.4.4.7 Effect of p-Hydroxyphenylglycine Dane salt

Preparation of mixed anhydride was tried by using different quantities of Dane salt from 1.05 to 1.22 moles. Better results were obtained by 1.05 moles (Table-9)

Table-9

Quantity of Dane salt in mole w.r.t. 7-APCA	Unreacted %7-APCA in reaction
1.05	1.07
1.07	4.1
1.10	3.90
1.12	1.20
1.16	1.15
1.20	3.77
1.22	2.3

3.4.4.8 Effect of temperature

Preparation of mixed anhydride was carried out at different temperature from –20 to –50°C. No adverse effect of temperature on the reaction up to –20°C but for safer side selected the temperature range between –35to –40°C Table-10

Table-10

Temperature of Mixed anhydride Preparation in °C	Unreacted %7-APCA in reaction
-20 to -25	4.17
-35 to -40	1.7
-45 to -50	2.3

3.4.4.9 Role of co-solvent in preparation of Cefprozil DMF solvate

Different co-solvents were tried in the preparation of Cefprozil solvate along with N,N-Dimethyl formamide, such as Isopropyl alcohol, ethyl acetate, dichloromethane and acetone. Acetone was found suitable co-solvent for the preparation of Cefprozil solvate in terms of good filtration rate and yield.

Quantity of acetone was explored from 3-25 times w.r.t.7-APCA. Three time acetone with 12 time N,N-Dimethyl formamide gives better results **Table-11**

Table -11

S.No.	DMF Vol / g 7-APCA	Co-solvent In times	w/w Yield of solvate w.r.t. 7-APCA
1	25.0	Isopropyl alcohol –10	1.0
2	10.0	Isopropyl alcohol -25	1.80
3	10.0	Acetone -25	1.70
4	12.0	Acetone -3	1.80
5	12.0	Dichloromethane -15	1.63
6	12.0	Ethyl acetate -15	1.72

3.4.4.10 Preparation of Cefprozil monohydrate from Cefprozil DMF Solvate

Cefprozil DMF Solvate which on desolvation in aqueous media presence of ethyl acetate and acetone gives Cefprozil monohydrate. Better results were obtained in the mixture of ethyl acetate and water. Desolvation of the Cefprozil solvate was carried out in different quantity of water from 1-6 times w.r.t. 7-APCA in presence ethyl acetate and acetone. The mixture of water (3.0 t) and ethyl acetate (1.80 t) is found to be suitable for better yield Table-12

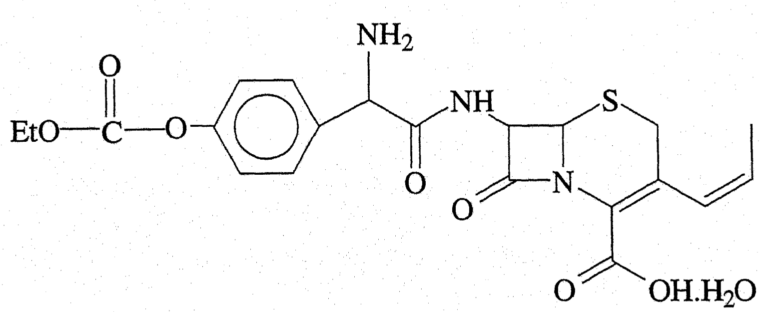
Table 12

S.No	D.M. Water Volume/gm 7-APCA	Co-solvent In times	w/w Yield of solvate w.r.t. 7-APCA
1	1.0	----	1.36 (low purity)
2	1.50	----	1.31
3	2.0	----	1.29
4	3.4	-----	1.20
5	4.0	------	0.90
6	6.0	------	0.50
7	3.0	Acetone (1.0 T)	1.3
8	1.5	Acetone (1.8 T)	1.3
9	3.0	(Ethyl acetate 1.8T)	1.3

3.4.5 Synthesis, isolation and characterization of impurities:

Regulatory authorities all over the world are becoming very stringent about the purity of an approved drug. Especially there is growing concern about the nature and level of impurities present in such molecules. US Pharmacopoeia specifies that the purity of Cefprozil[17] should be between 90 to 105 %. However, most of the reported methods are associated with the formation of varying amounts of impurities and hence there is need to identify the cause of formation and characterization of impurities

Mainly two process impurities were observed in the Cefprozil during its synthesis in the range of 0.2 to 1.5% , the molecular weight of these impurities were found by LC-MS and from the molecular weight the structure of the impunities were illustrated as shown below structure (26) and (27) . Efforts were made for the synthesis of these impurities.



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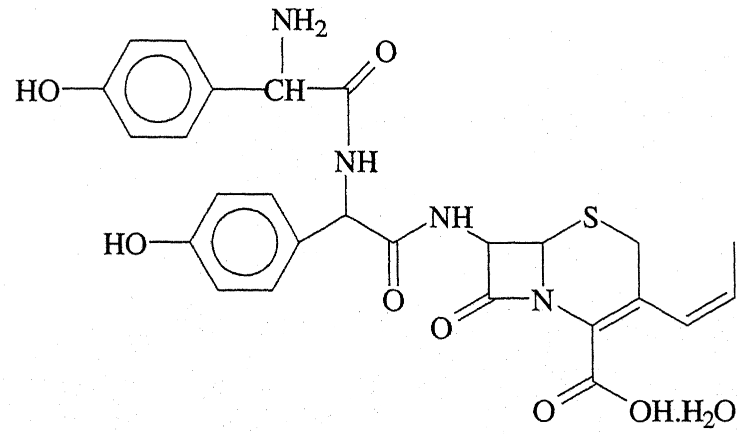

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Impurity (26) was synthesized from 7-amino-3- [(Z/E)-propenyl]-3-cephem-4-carboxylic acid and N-substituted-α-amino-p-hydroxy phenyl acetic acid or its salt (Dane salt) by using excess quantity of ethylchloroformate and N-methyl morpholine .as process described in example (5)

Similarly the second impurity (27) was synthesized from silyl protected Cefprozil and mixed anhydride of N-substituted-α-amino-p-hydroxy phenyl acetic acid salt (Dane salt). Detailed process was mentioned in experimental section as example (6)

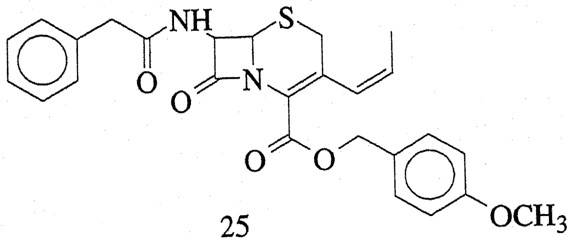
3.5 Conclusion

In summary, the present work provides a highly selective method for preparation of Cefprozil in high yield and high purity, substantially free of impurities, which is simple, convenient and cost-effective and more importantly does not suffer from the limitations associated with the reported methods.

3.6 Experimental:

Preparation of 4-methoxybenzyl 7-phenylacetamido-3-[(Z/E)-propenyl]-3-cephem-4-carboxylate (25.)

To a suspension of 4-methoxybenzyl 7-phenylacetamido-3-chloromethyl-3-cephem-4-carboxylate (100 g, 0.205 mol) in dimethyl formamide (200 ml), NaBr (24 g, 0.233 mol), triphenyl phosphine (58 g, 0.220 mol) and dichloromethane (100 ml) were added and stirred at 25 to 27$^{\circ C}$ for 2 hrs. The reaction mixture is cooled to 10°Cand dichloromethane (1000 ml) was added followed by addition of aqueous solution of sodium hydroxide (9.6 g in 80 ml) in 45 to 60 minutes at 5 to 7°C. After stirring for 2 hrs, water (1000 ml) was added to the reaction mixture and stirred for 15 min at 10 to 12°C. The organic layer was separated and washed with water (1000 ml). The organic layer was added to a flask charged with 20% w/v aqueous NaCl solution (1200 ml) and isopropyl alcohol (200 ml) cooled to 5-10°C. A solution of aq. NaOH (1.37 g in 35 ml) was added in 10-15 min followed by acetaldehyde (140 ml, 3.2 mol) in 45 min at 5-10°C, stirred for 4 hrs at 5 to 10°C and 10% v/w hydrochloric acid (10.4 ml) was added and stirred for 15 min at 10°C. The organic layer was separated, concentrated to almost no solvent and isopropyl alcohol (400 ml) was added, heated to 50 to 52°C with stirring for 30 min, cooled and filtered the solid. The wet solid was washed with chilled isopropyl alcohol (100 ml) and dried under vacuum at 35-40°C till moisture content was not more than 1% (yield 49 g, 50%, Z/E ratio 92/8, by HPLC)



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^{1}H NMR Spectrum ($CDCl_3$, δ in ppm):

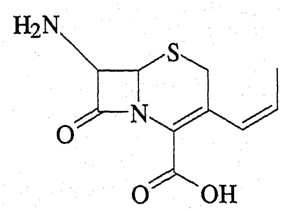
1.51–1.53 d (3H, (Z)-$\underline{CH_3}$), 1.75 d (3H, (E)-CH_3), 3.18-3.79 m (4H, -S-$\underline{CH_2}$ and Ph$\underline{CH2}$), 3.79 s (3H, -O$\underline{CH_3}$),4.95-5.13 m (3 H , CO_2-$\underline{CH_2}$ and –COCH-$\underline{CH}$-), 5.59—5.82 m (1H ,-$\underline{CH}$=CH(CH_3) and –CO$\underline{CH}$-CH-)), 6.03-6.09 d(1H, ,-CH=$\underline{CH}$(CH_3)),6.86 d (2H, benzene-H)7.25-7.36 m (7H, benzene –H).

IR (KBr) cm^{-1}: 2600-3304, (OH,NH,NH_2), 1776,(β -lactam ,-C=O),1714-1722 (Amide , -C=O), 1612 ,(Carboxylic , -C=O)

Preparation of 7-amino-3-[(Z/E)-propenyl]-3-cephem-4-carboxylic acid (16)

4-Methoxybenzyl 7-phenylacetamido-3-[(Z/E)-propenyl]-3-cephem-4-carboxylate **25** (50 g, 0.0954 mol) was dissolved in dichloromethane (500 ml), trimethylsilyl chloride (5 ml) was added at 0°C, stirred for 10 min. The reaction mass is cooled to –55°C, N, N-dimethylaniline (29.11 g, 0.241 mol) was added at –50 to –55°C and stirred for 10 min. PCl_5 (41.4 g, 0.199 mol) was added at –40 to –55°C and stirred at –35 to –40°C for 4 hrs. Methanol (100 ml) was added in 45-50 min at –35 to –20°C and stirred for 3 hrs. A 20 % w/v aq. NaCl solution (400 ml) was added and stirred for 15 min at 10oC. The layers were separated, aqueous layer was extracted with dichloromethane (100 ml) and the combined organic layer was concentrated to one

fifth of its original volume. Trifluoroacetic acid (50 ml) was added to the concentrated organic layer at 20 to 22°C in about 20-30 min and stirred for 8 to 10 hrs at 17 to 20°C. Water (400 ml) was added at 18 to 20°C and stirred for 20 min. After separating the layers, the organic layer is extracted with water (100 ml). Activated carbon (2 g), and EDTA (0.5 g) were added to the aqueous layer, stirred for 15 min at 20°C, filtered through celite and washed with water (100 ml). The pH of the filtrate was adjusted to 3.5 by adding 20% w/v aq. NaOH solution over a period of 60 min, the slurry is stirred, filtered and solid was washed with water (2 x 100 ml), acetone (2 x 100 ml); dried under vacuum at 40-45°C till the moisture content was not more than 0.5% w/w (yield 19 g; 76%; purity 97.39%, Z/E ratio 89/11 by HPLC).



16

^{1}H NMR Spectrum (DMSO-d_6, δ in ppm):

1.61 d (3H, (E)-$\underline{CH_3}$), 1.77 d (3H, (Z)-CH_3), 3.37-3.81 m (2 H, -S-$\underline{CH_2}$), 4.74 d (1 H , CO.CH-$\underline{CH}$), 4.98 d (1 H , CO.$\underline{CH}$-CH), 5.99—6.14 m (1H ,-$\underline{CH}$=CH(CH_3)), 6.60-6.68 d(1H, ,-CH=$\underline{CH}$(CH_3)).

IR (KBr) Cm^{-1}: 3230,1805,1620, 1535

Theoretical mass :240

Mass : 239 (M-H)

Preparation of 7-[D-α-amino-α-(4-hydroxyphenyl) acetamido]-3-(1-propen-1-yl)-3-cephem-4-carboxylic acid (11)

Step A

To a mixture of methylene chloride (125 ml) and N,N-dimethyl formamide (85 ml), cooled to 20-25°C, was added a solution of N-methylmorpholine in dichloromethane (0.25 g, 0.0025 mole in 5 ml) and ethyl chloroformate in dichloromethane (12.72 g, 0.117 mole in 10 ml) under stirring. The resulting solution was cooled to –40° to –50°C and potassium D-N-(2-methoxycarbonyl-1-methylvinyl)-α-amino-α-(4-hydroxylphenyl) acetate (33.14 g, 0.11 mol) was added to it. The suspension is agitated at –40° to –35°C for 120 minutes. The reaction mass which was a solution of mixed anhydride product was cooled to –70 °C for condensation.

Step B

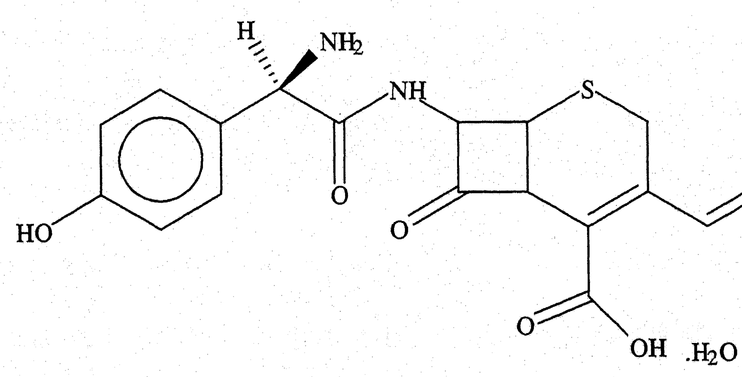
7-APCA (25 g, 0.104 mole) was added to methylene chloride (100 ml) at 25° to 30°C followed by addition of N,O-bis-trimethylsilyl acetamide (0.125 g, 0.0006 mole), trimethylchlorosilane (9.3 g, 0.086 mole) and hexamethyl disilazane (13.4 g, 0.083 mole). The reaction mass was heated to reflux temperature and refluxed for 4 hours to obtain silylated 7–amino-3-(propan-1-yl)-3-cephem-4-carboxylic acid (7-APCA)compound.

Step C

To a solution of the mixed anhydride product of procedure 1A, cooled to –70 °C, was added with stirring, a cooled solution of the disilylated 7-APCA as prepared by procedure 1B. The reaction mixture was stirred at –50° to –40°C and monitored by

HPLC till quantitative conversion to the silylated Cefprozil was achieved. The reaction time was about 4 hours. The resulting reaction mass was added to a mixture of 85 ml water and 20 ml concentrated hydrochloric acid maintained at –40° to –20°C. The temperature of the reaction mass was raised to 5 to10°C and the pH of the solution was adjusted to 0.5 with concentrated hydrochloric acid. The reaction mass was stirred for 30 minutes and the layers are separated. The aqueous was diluted with acetone (75 ml) followed by the addition of 2.5 g of activated carbon and sodium dithionite (0.25 g) to remove the colored solid impurities. The reaction temperature was maintained at 10 to15°C.

The aqueous solution containing Cefprozil was added to prechilled DMF (300 ml) at 15-18°C. The reaction mixture was basified to pH 6 t0 6.5 by ammonia solution. The solid was stirred for 2.0 h at 20-25°C. The DMF solvate of Cefprozil was filtered off and washed with DMF (50 ml) followed by ethyl acetate (500ml) The wet DMF solvate without drying is desolvated by stirring with a mixture of demineralised water (75 ml) and ethyl acetate (45 ml) for 60 minutes. The product was filtered and dried to give Cefprozil monohydrate in the form of an off white to pale yellow crystalline powder. Yield: 30 gms, % Yield: 70.7, Purity: 101.2%, Total impurities: 0.45%



11

^{1}H NMR Spectrum (DMSO-d_6, δ in ppm):

1.61–1.64 d (3H, (Z)-$\underline{CH_3}$), 1.69-1.172 d (3H, (E)-CH_3), 3.26-3.57 m (2H, -S-$\underline{CH_2}$), 4.71 s (1H, -CO-$\underline{CH}$-NH_2),4.91-d (1 H , CO-CH-$\underline{CH}$) 4.93 d (1 H , CO-$\underline{CH}$-CH) , 5.37—5.46 m (1H ,-CH=$\underline{CH}$(CH_3)), 6.23-6.28 d (1H, ,-$\underline{CH}$=CH(CH_3)),6.66 -6.75 m(2H, benzene-H Ortho to –CH-NH_2), 7.17 -7.25 m (2H, benzene-H para to CH-NH_2),8.84-8.94 m (2H,-NH_2).

IR (KBr) Cm^{-1}: 3546,1758,1683,1563,1515,1461,1396,1343,1312 1269,1234.

Theoretical mass :389

Mass : 390 (M+H),407 (M+H),412 (M+H)

Preparation of 7-[D-α-amino-α-(4-hydroxyphenyl) acetamido]-3-(1-propen-1-yl)-3-cephem-4-carboxylic acid (11)

Step A

Methylene chloride (125 ml) was cooled to 20-25°C, and a solution of N-methylmorpholine in dichloromethane (0.25 g, 0.0025 mole in 5 ml) and ethyl chloroformate in dichloromethane (12.72 g, 0.117 mole in 10 ml)were added under stirring. The resulting solution was cooled to –40° to –50°C and potassium D-N-(2-methoxycarbonyl-1-methylvinyl)-α-amino-α-(4-hydroxylphenyl) acetate (33.14 g, 0.11 mol) is added to it. The suspension is agitated at –40° to –35°C for 90 minutes. N,N-dimethyl formamide (85 ml), cooled to –70°C, was added and the suspension was further agitated for 30 minutes. The reaction mass which was a solution of mixed anhydride product was cooled to –70 °C for condensation.

Step B

7-APCA (25 g, 0.104 mole) was added to methylene chloride (100 ml) at 25° to 30°C followed by addition of N,O-bis-trimethylsilyl acetamide (0.125 g, 0.0006 mole), trimethylchlorosilane (9.3 g, 0.086 mole) and hexamethyldisilazane (13.4 g, 0.083 mole). The reaction mass was heated to reflux temperature and refluxed for 4 hours to obtain silyated 7–amino-3-(propan-1-yl)-3-cephem-4-carboxylic acid (7-APCA)compound.

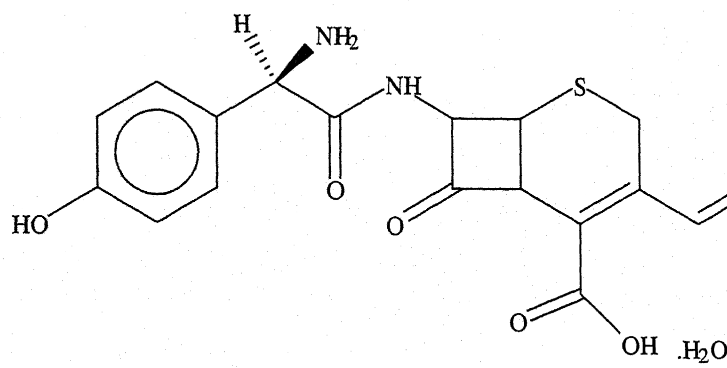
Step C

To a solution of the mixed anhydride product of procedure 2A, cooled to –70 °C, was added with stirring, a cooled solution of the disilylated 7-APCA as prepared by procedure 2B. The reaction mixture was stirred at –50° to –40°C and monitored by HPLC till quantitative conversion to the silylated Cefprozil was achieved. The reaction time was about 4 hours. The resulting reaction mass was added to a mixture of 85 ml water and 20 ml concentrated hydrochloric acid maintained at –40° to –20°C. The temperature of the reaction mass was raised to 5° to10°C and the pH of the solution was adjusted to 0.5 with concentrated hydrochloric acid. The reaction mass was stirred for 30 minutes and the layers are separated. The aqueous layer was diluted with acetone (75 ml) followed by the addition of 2.5 g of activated carbon and sodium dithionite (0.25 g) to remove the colored solid impurities. The reaction temperature was maintained at 10° to15°C.

The aqueous solution containing Cefprozil as obtained above was converted to its DMF solvate as per example (3). The wet DMF solvate without drying was desolvated by stirring with a mixture of demineralised water (75 ml) and ethyl

acetate (45 ml) for 60 minutes. The product was filtered and dried to gave Cefprozil monohydrate in the form of an off white to pale yellow crystalline powder.

Yield: 33.4 gms, % Yield: 78.7, Purity: 101.3%, Total impurities: 0.5%



[1]H NMR Spectrum (DMSO-d_6, δ in ppm):

1.61–1.64 d (3H, (Z)-$\underline{CH_3}$), 1.69-1. 72 d (3H, (E)-CH_3), 3.26-3.57 m (2H, -S-$\underline{CH_2}$), 4.71 s (1H, -CO-<u>CH</u>-NH_2),4.91-d (1 H , CO-CH-<u>CH</u>) 4.93 d (1 H , CO-<u>CH-</u>CH) , 5.37—5.46 m (1H ,-CH=<u>CH</u>(CH_3)), 6.23-6.28 d (1H, ,-<u>CH</u>=CH(CH_3)),6.66 -6.75 m(2H, benzene-H Ortho to –CH-NH_2), 7.17 -7.25 m (2H, benzene-H para to CH-NH_2),8.84-8.94 m (2H,-NH_2).

IR (KBr) Cm^{-1}: 3546,1758,1683,1563,1515,1461,1396,1343,1312 1269,1234.

Theoretical mass :389

Mass : 390 (M+H),407 (M+H),412 (M+H)

Example –5

(6R)-3-((1Z)prop-1-enyl)-6-[2-amino-2-(4-ethoxycarbonyloxyphenyl)acetylamino]--5-oxo-6aH-azetidino[2,1-b]1,3-thiazine-4-carboxylic acid .(Carbonate impurity)

Step A

Methylene chloride (125 ml) was cooled to 20-25°C, and a solution of N-methylmorpholine in dichloromethane (10.52 g, 0.104mole in 15 ml) and ethyl chloroformate in dichloromethane (24.86 g, 0.229mole in 10 ml) were added under stirring. The resulting solution was cooled to –40° to –50°C and potassium D-N-(2-methoxycarbonyl-1-methylvinyl)-α-amino-α-(4-hydroxylphenyl) acetate (33.14 g, 0.11 mol) was added to it. The suspension was agitated at –40° to –35°C for 90 minutes. N,N-dimethyl formamide (85 ml), cooled to –70°C, was added and the suspension was further agitated for 30 minutes. The reaction mass which was a solution of mixed anhydride product is cooled to –70 °C for condensation.

Step B

7-APCA (25 g, 0.104 mole) was added to methylene chloride (100 ml) at 25° to 30°C followed by addition of N,O-bis-trimethylsilyl acetamide (0.125 g, 0.0006 mole), trimethylchlorosilane (9.3 g, 0.086 mole) and hexamethyldisilazane (13.4 g, 0.083 mole). The reaction mass was heated to reflux temperature and refluxed for 4 hours to obtain silyated 7–amino-3-(propan-1-yl)-3-cephem-4-carboxylic acid (7-APCA)compound.

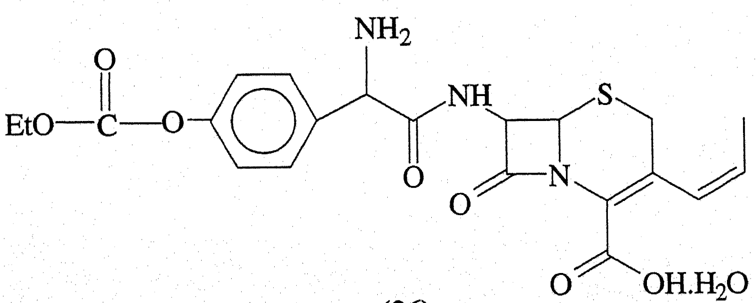
Step C

To a solution of the mixed anhydride product of procedure 2A, cooled to –70 °C, was added with stirring, a cooled solution of the disilylated 7-APCA as prepared by procedure 2B. The reaction was stirred at –50° to –40°C and monitored by HPLC till quantitative conversion to the silylated Cefprozil was achieved. The reaction time was about 4 hours. The resulting reaction mass was added to a mixture of 85 ml water and 20 ml concentrated hydrochloric acid maintained at –40° to –20°C. The

temperature of the reaction mass was raised to 5° to10°C and the pH of the solution was adjusted to 0.5 with concentrated hydrochloric acid. The reaction mass was stirred for 30 minutes and the layers are separated. The aqueous layer was diluted with acetone (75 ml) followed by the addition of 2.5 g of activated carbon and sodium dithionite (0.25 g) to remove the colored solid impurities. The reaction temperature was maintained at 10° to15°C.

The aqueous solution containing impurity rich Cefprozil as obtained above was converted to its DMF solvate as per example(3) . The wet DMF solvate without drying was desolvated by stirring with a mixture of demineralised water (75 ml) and ethyl acetate (45 ml) for 60 minutes. The product was filtered was purified by the column chromatography using the resin XAD –1180 at pH 6.0-6.5. The elute then acidified to pH 2.8 to 3.2 by 10% hydrochloric acid solid formation takes place .The solid was filtered off and dried under vacuum ,16 .0 gm of title impurity

Was collected in the pure form .



(26)

Molecular formula: $C_{21}H_{23}O_7N_3S$

Molecular weight : 461.4

Mass : 462.4(M+H),479.2 (M+H), 484 (M+H)

^{1}H NMR Spectrum (DMSO-d_6, δ in ppm):

1.25-1.32 t (3H, $-OCH_2-CH_3$), 1.62-1.66 d (3H, -CH=CH ($\underline{CH_3}$)), 3.51-3.56 dd (2H, -S-$\underline{CH2}$), 4.19-4.26 q (2H, $-O\underline{CH_2}CH_3$), 4.46 d (1 H , CO-CH-$\underline{CH}$) , 4.55 d (1 H , CO-$\underline{CH}$-CH) , 5.1 s (1H, -CO-$\underline{CH}$-NH_2), 5.32—5.41 m (1H ,-CH=$\underline{CH}$(CH_3)), 5.71-5.75 d (1H, ,-$\underline{CH}$=CH(CH_3)), 7.21 –7.25 m (2H, benzene-H *Ortho* to –CH-NH_2), 7.37 -7. 41 m (2H, benzene-H *Para* to CH-NH_2),8.84-8.94 d (2H,-$\underline{NH_2}$).

Example –6

3-((1Z)prop-1-enyl)-6-{2-[2-amimo-2-(4-hydroxyphenyl)acetylamino]-2-(4-hydroxyphenyl)acetylamino}-5-oxo-2H,6H.6aH-azetidino[2,1-b]1,3-thiazine-4-carboxylic acid (N-Glycil impurity).

Step A

Methylene chloride (125 ml) was cooled to 20-25°C, and a solution of N-methylmorpholine in dichloromethane (0.25 g, 0.0025 mole in 5 ml) and ethyl chloroformate in dichloromethane (7.47 g, 0.0688 mole in 10 ml) were added under stirring. The resulting was cooled to –40° to –50°C and potassium D-N-(2-methoxycarbonyl-1-methylvinyl)-α-amino-α-(4-hydroxylphenyl) acetate (20.07 g,.0.0662mol) was added to it. The suspension was agitated at –40° to –35°C for 90 minutes. N,N-dimethyl formamide (85 ml), cooled to –70°C, was added and the suspension was further agitated for 30 minutes. The reaction mass which was a solution of mixed anhydride product was cooled to –70 °C for condensation.

Step B

Cefprozil (25 g, 0.0614 mole) was added to methylene chloride (100 ml) at 25° to 30°C followed by addition of N,O-bis-trimethylsilyl acetamide (0.125 g, 0.0006 mole), trimethylchlorosilane (10.94 g, 0.100 mole) and hexamethyl disilazane (15.81 g, 0.098mole). The reaction mass was heated to reflux temperature and refluxed for 4 hours to obtain silyated 7-[D-α-amino-α-(4-hydroxyphenyl) acetamido]-3-(1-propen-1-yl)-3-cephem-4-carboxylic acid (Cefprozil) compound.

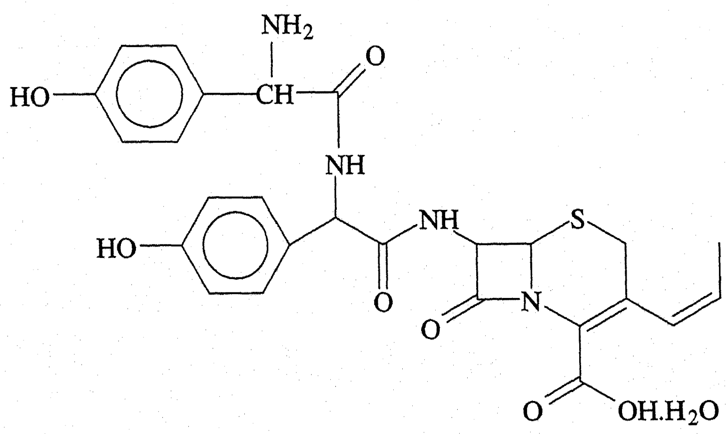
Step C

To a solution of the mixed anhydride product of procedure 2A, cooled to –70 °C, was added with stirring, a cooled solution of the disilylated Cefprozil as prepared by procedure 2B. The reaction mixture was stirred at –50° to –40°C and monitored by HPLC till quantitative conversion to the silylated Cefprozil was achieved. The reaction time was about 4 hours. The resulting reaction mass was added to a mixture of 85 ml water and 20 ml concentrated hydrochloric acid maintained at –40° to –20°C. The temperature of the reaction mass was raised to 5° to10°C and the pH of the solution is adjusted to 0.5 with concentrated hydrochloric acid. The reaction mass was stirred for 30 minutes and the layers are separated. The aqueous layer was diluted with acetone (75 ml) followed by the addition of 2.5 g of activated carbon and sodium dithionite (0.25 g) to remove the colored solid impurities. The reaction temperature was maintained at 10° to15°C.

The aqueous solution containing N-glycl Cefprozil as obtained above was converted to its DMF solvate as per the method given in example (3). The wet DMF solvate of

N-glycl Cefprozil without drying was desolvated by stirring with a mixture of demineralised water (75 ml) and ethyl acetate (45 ml) for 60 minutes. The product was filtered and dried to give title impurity (**27**) in the form of an off white to crystalline powder.

Yield: 20 gm.



27

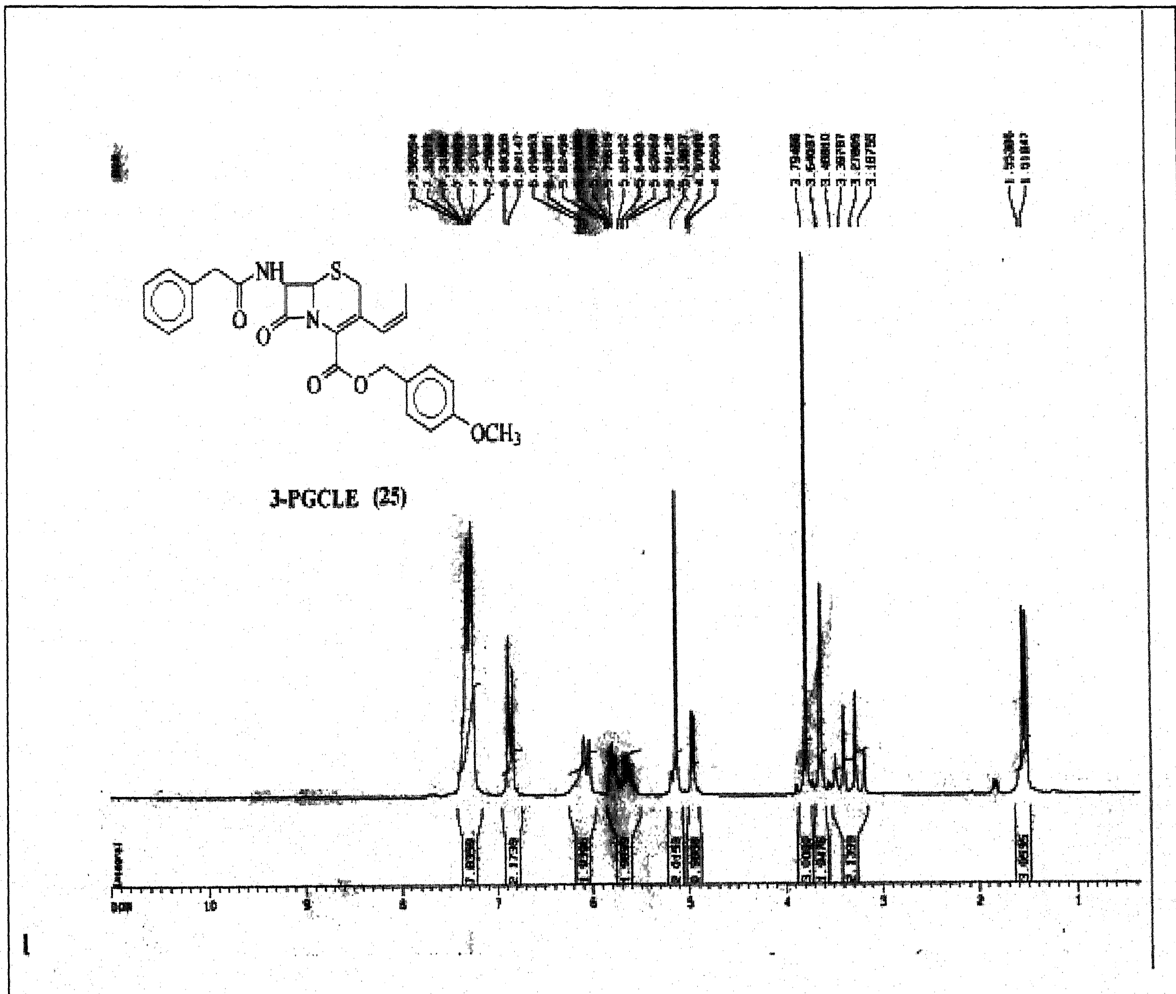
Molecular formula: $C_{26}H_{26}O_7N_4S$

Molecular weight : 538.4

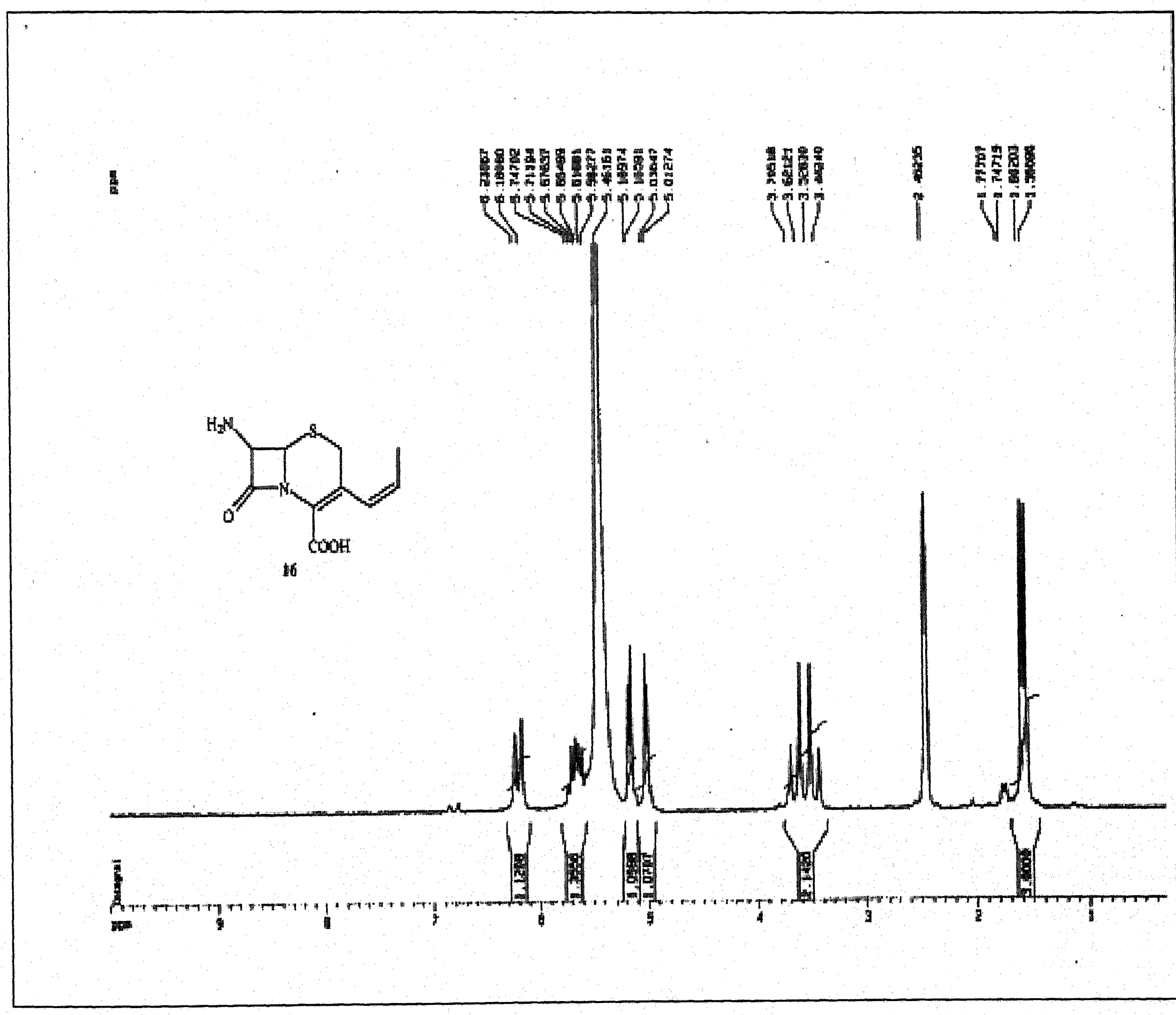
Mass : 539.4(M+H),561.3 (M+H), 562.2 (M+H)

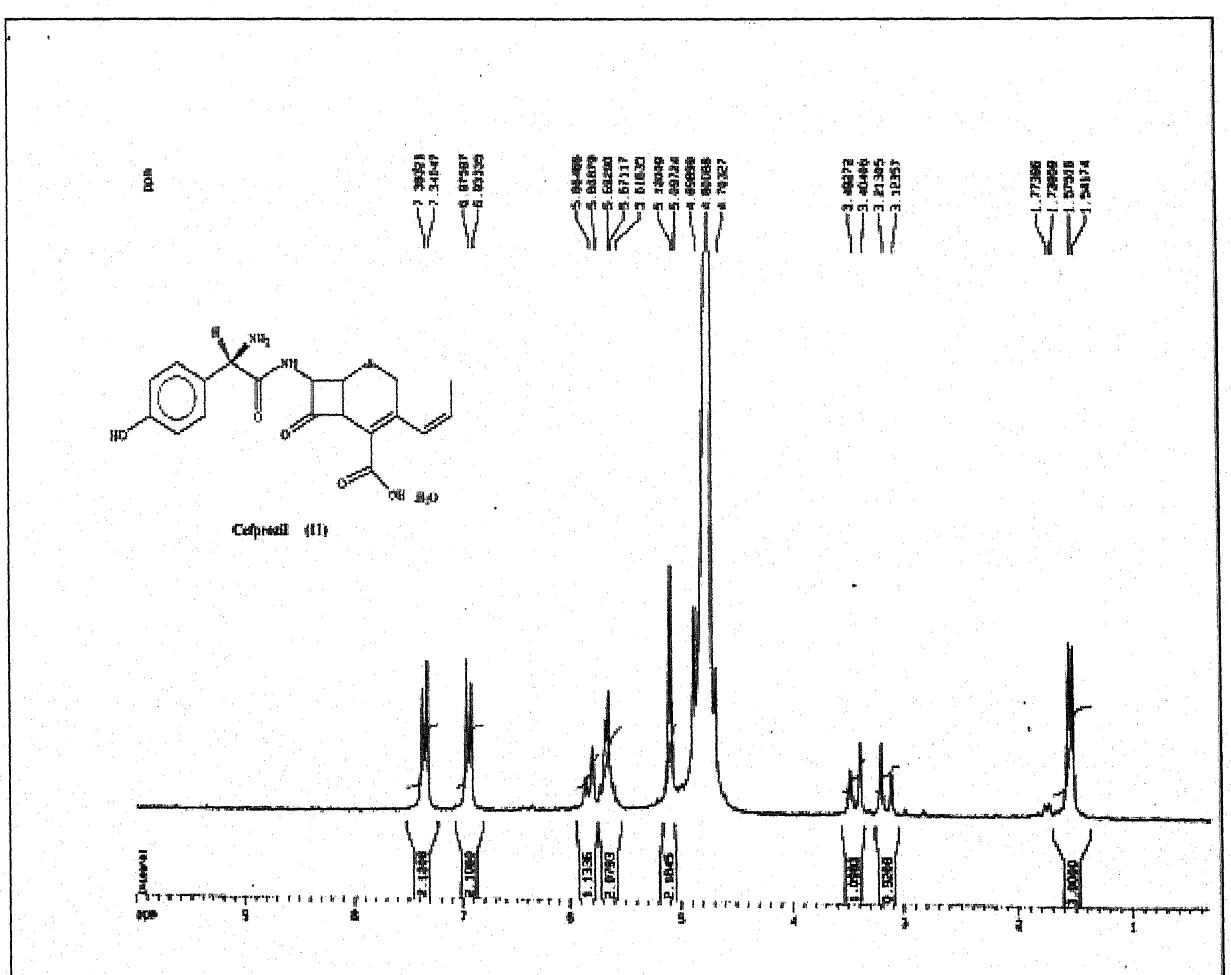
^{1}H NMR Spectrum (DMSO-d_6, δ in ppm):

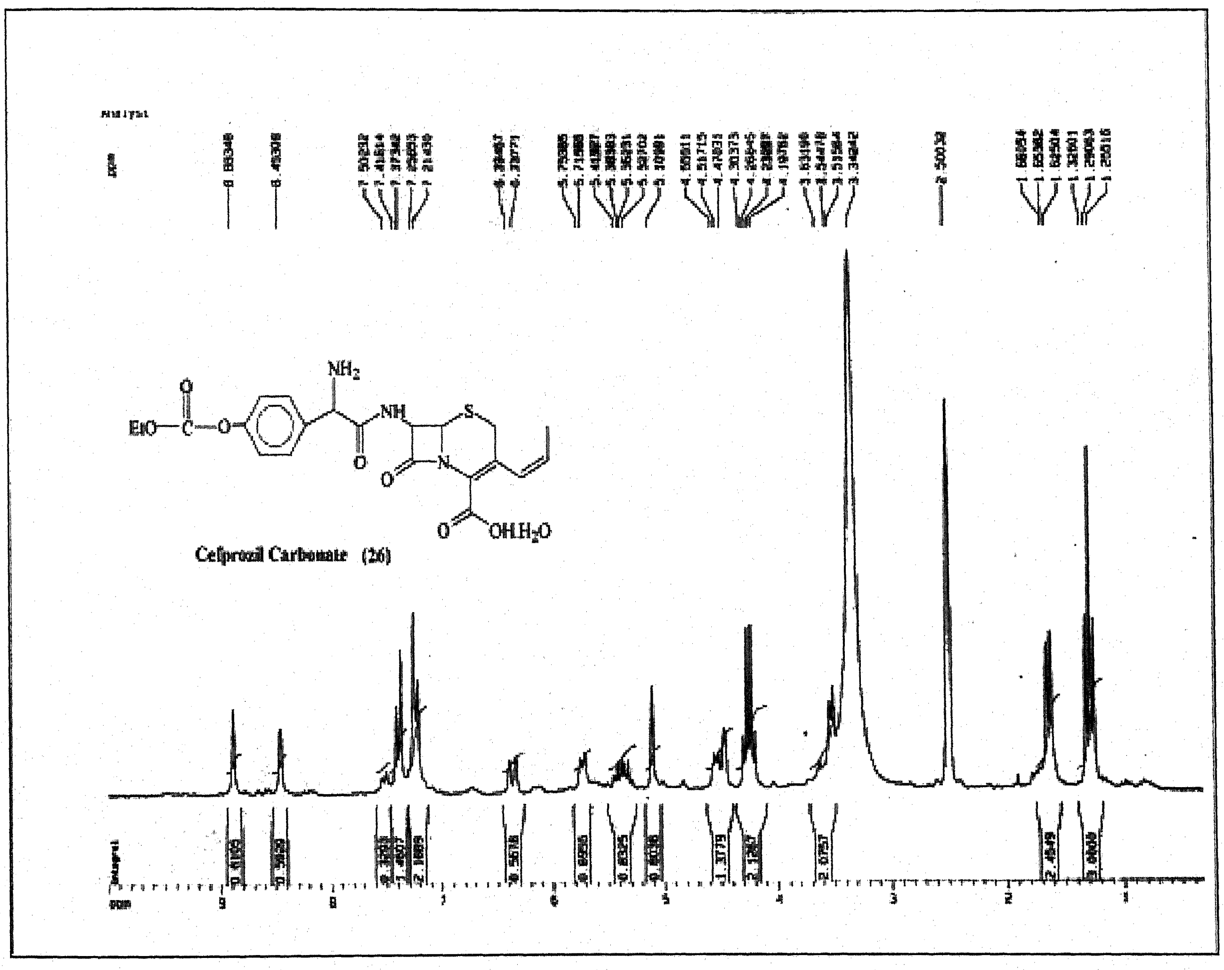
1.66 d (3H, -CH=CH (CH$_3$)), 3.28-3.59 m (2H, -S-CH2), 4.71 d (1 H , CO-CH-CH) , 4.55 d (1 H , CO-CH-CH) , 4.94 s (1H, -CO-CH-NH$_2$), 5.34—5.55 m (1H ,-CH=CH(CH$_3$)), 6.25. –6.31 d (1H, ,-CH=CH(CH$_3$)), 6.67–6.77 m (4H, benzene-H *Ortho* to –CH-NH$_2$), 7.18 -7. 26 m (4H, benzene-H *Para* to CH-NH$_2$),8.85-8.96 d (2H,-NH$_2$).



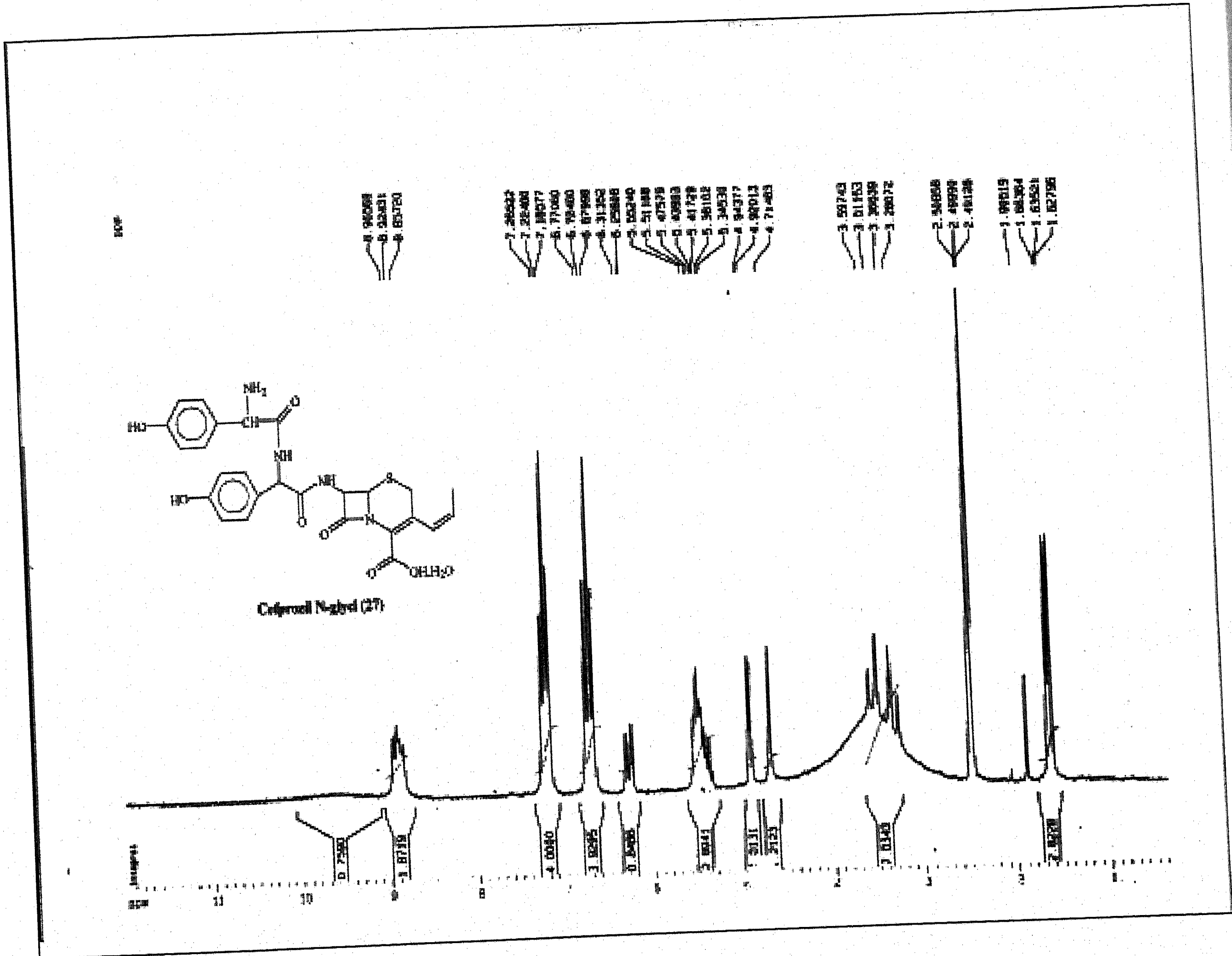
3-PGCLE (25)







Cefprozil Carbonate (26)



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Chapter –IV

A Novel process for the preparation of Cefepime.

4.1 Introduction

Many of the recently introduced[2] Cephalosporin have a common structural feature in that they have a 2-aminothiazolylacetamido group in the 7- side chain with an α-oxyimino substitution. These include Cefotaxime, Ceftriaxone, Ceftazidime, Cefmenoxime and Ceftazidime, which are characterized by their excellent activity against Enterobacteriaceae. They are however relatively weak in anti-staphylococcal activity as compared to older cephalosporins such as cephalothin and cefazolin. Among the new family of cephalosporins, only Ceftazidime exhibits a potent anti-pseudomonal activity but unfortunately it is less active than other members of this group of Cephalosporins against Staphylococci .the substantial improvement in anti- Staphylococcal activity was achieved by replacing the 2-carboxy-2-ppropoxyimino group in the side chain with an alkoxyimino substitution. Thus a series of α-7 alkoxyimino derivatives having quaternized ammonium group in the 3- side chain were prepared . Among them , 7-[2-(2-aminotiazol-4yl)-2-(z)-methoxy-iminoacetamido]-3-[(1-methyl-1-pyrolidino)-methyl] ceph-3-em-4carboxylate (Cefepime) was found to be most promising in view of its antimicobial spectrum and other other biological properties .

A large number of Cephalosporin antibiotics are known and are widely used in the treatment of bacterial infection. Cefepime (BYM-28142) is a fourth generation injectable Cephalosporin antibiotic with an improved stability to β-lactamases and a broader spectrum of activity than third generation Cephalosporin, such as Cefotaxime and Ceftazidime. Cefepime was discovered and developed by Bristol-

Myers Company, USA. In literature various routes have been described to prepared Cefepime hydrochloride.

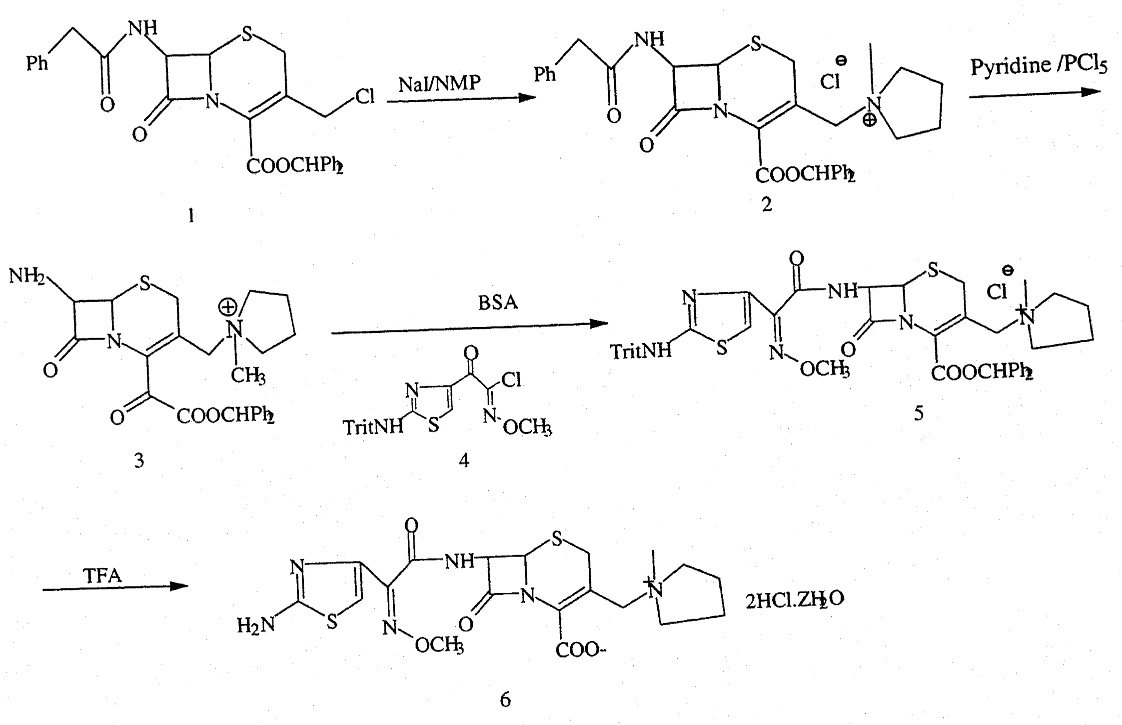
Cefipime is a commercially valuable and therapeutically useful oral Cephalosporin antibiotic effective in controlling diseases caused by a wide variety of Gram positive and Gram-negative microorganisms.

4.2 Literature methods for the preparation of Cefepime

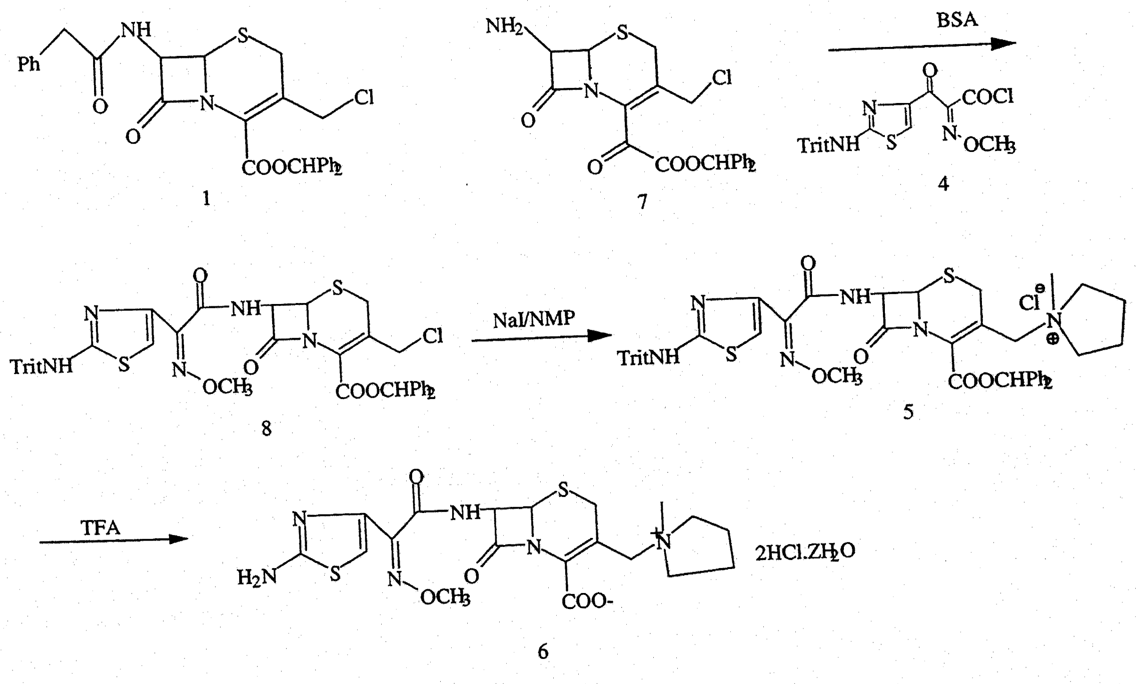
Aburaki, et al.[1] were first reported the synthesis of Cefepime hydrochloride._They describes a method for preparing Cefepime hydrochloride starting from benzahydryl-3-hydroxymethyl-7-phenylacetamido-3-cephem-4-carboxylate which on reaction with sodium iodide and N- methyl pyrrolidine followed by amide linkage cleavage and condensation with (Z)-2-methoxyimino-2-(2-tritylaminothiazol-4-l)acetyl chloride affords the protected ester. The final step involves deprotection of protecting group and treatment with hydrochloric acid to give Cefepime hydrochloride –scheme **4.1**

Alternatively Cefepime hydrochloride prepared by Abukari et al. involves condensation with (Z)-2-methoxyimino-2-(2-tritylaminothiazol-4-yl)acetyl chloride with benzahydryl-3-hydroxymethyl-7-phenylacetamido-3-cephem-4-carboxylate followed by reaction with sodium iodide and N- methyl pyrrolidine. The final step involves deprotection of protecting group and treatment with hydrochloric acid to give Cefepime hydrochloride (**6**).

Scheme 4.1

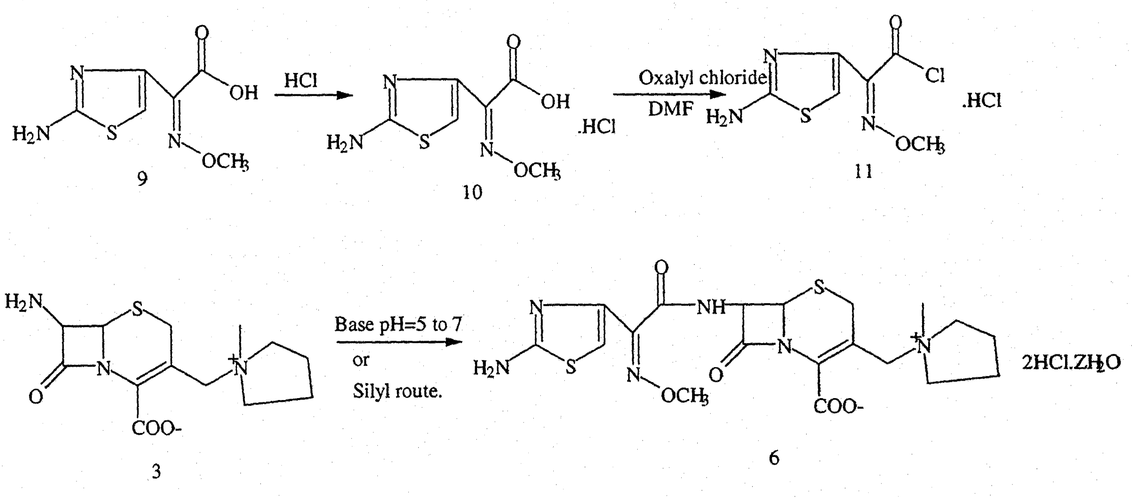

NaI/NMP
Pyridine /PCl5
BSA
TFA
2HCl.ZH2O

Scheme 4.2


BSA
NaI/NMP
TFA
2HCl.ZH2O

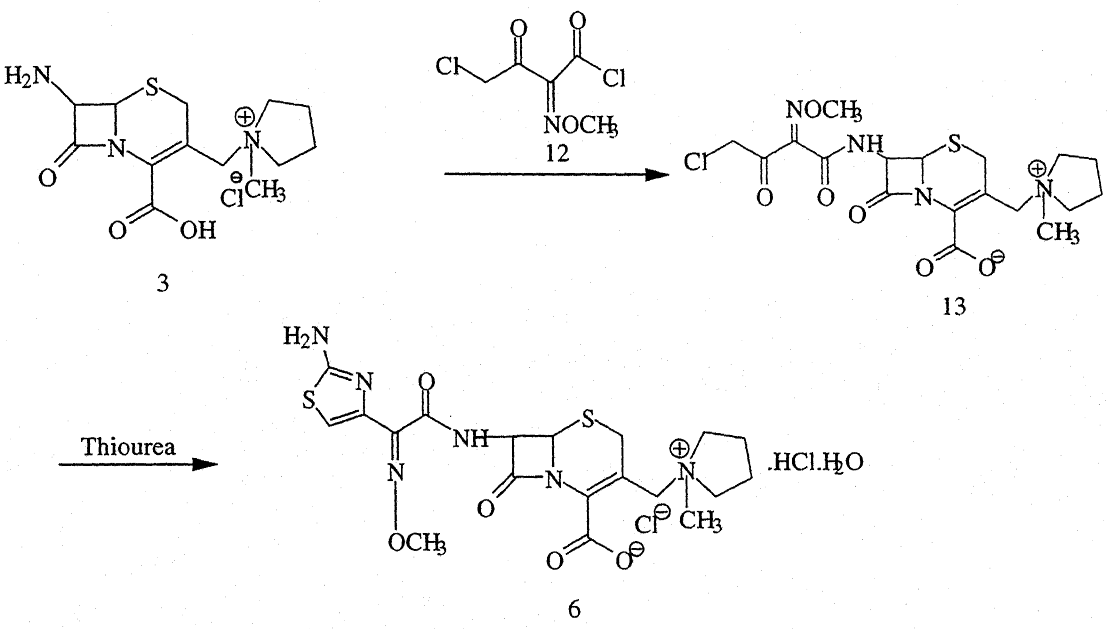
Gary and John [3,4] described a method for preparing Cefepime by the aqueous and anhydrous acylation of 7-amino-3-[(1-methyl-1-pyrrolidino)-methyl]ceph-3-em-4-carboxylate with syn isomer of 2-(2-aminothiazol-4-yl)-2-methoxy-imino acetyl chloride hydrochloride as shown in scheme **4.3**

Scheme 4.3



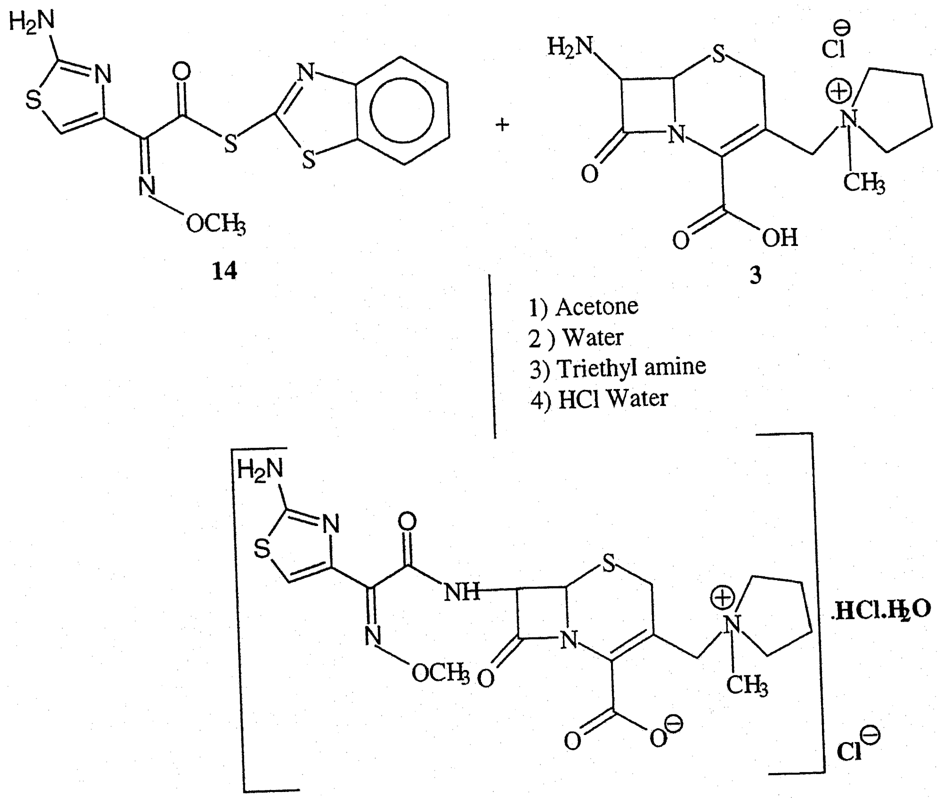
Ludescher J., Wolf S.[4] describes method for preparing Cefepime hydrochloride, by reacting the 4-chloro-2-methoxyimino-3-oxo-butyryl chloride with 7-amino-3-[(1-methyl-1-pyrrolidino)-methyl]ceph-3-em-4-carboxylate followed by Cyclisation with thiourea and treatment with hydrochloric acid as described in scheme **4.4** . Similarly it discloses the preparation of Cefepime hydrochloride from Cefotaxime.

Scheme 4.4



The same patent discloses the method for preparation of Cefepime hydrochloride by using (Z)-(2-aminothiazol-4-yl)methoxy-iminoacetic acid-2-mercaptobenzothiazolylester in acetone water solvent system.

Scheme 4.5



Cefepime hydrochloride

6

The above reported process having following limitation

1. Cefepime hydrochloride synthesis long and cumbersome synthetic steps involved in it, which increases the cost of synthesis.
2. The process utilizes anhydrous condition, which is difficult to achieve industrially, and adds to cost due to use of silylating agents.

3. The product obtained by above processes are associated with Δ^{2} and anti isomers of Cefepime.

4.2 Objective and Strategy

The main objective of the present work is to provide a novel intermediate useful for the preparation of Cefepime. Another objective of the present invention is to provide an improved process for the preparation of Cefipime of the formula (I), which is operationally simple in good yield and high purity.

Because of its therapeutic usefulness and efficient broad spectrum of activity, there is always a need for an improved synthetic process which would result in a product with high purity and yield, with minimum level of impurities, preferably absent, coupled with ease of operation and, more importantly, with low production cost.

The Cefepime hydrochloride was prepared by two routes, infringing cost effective and non-infringing and their industrially feasible process.

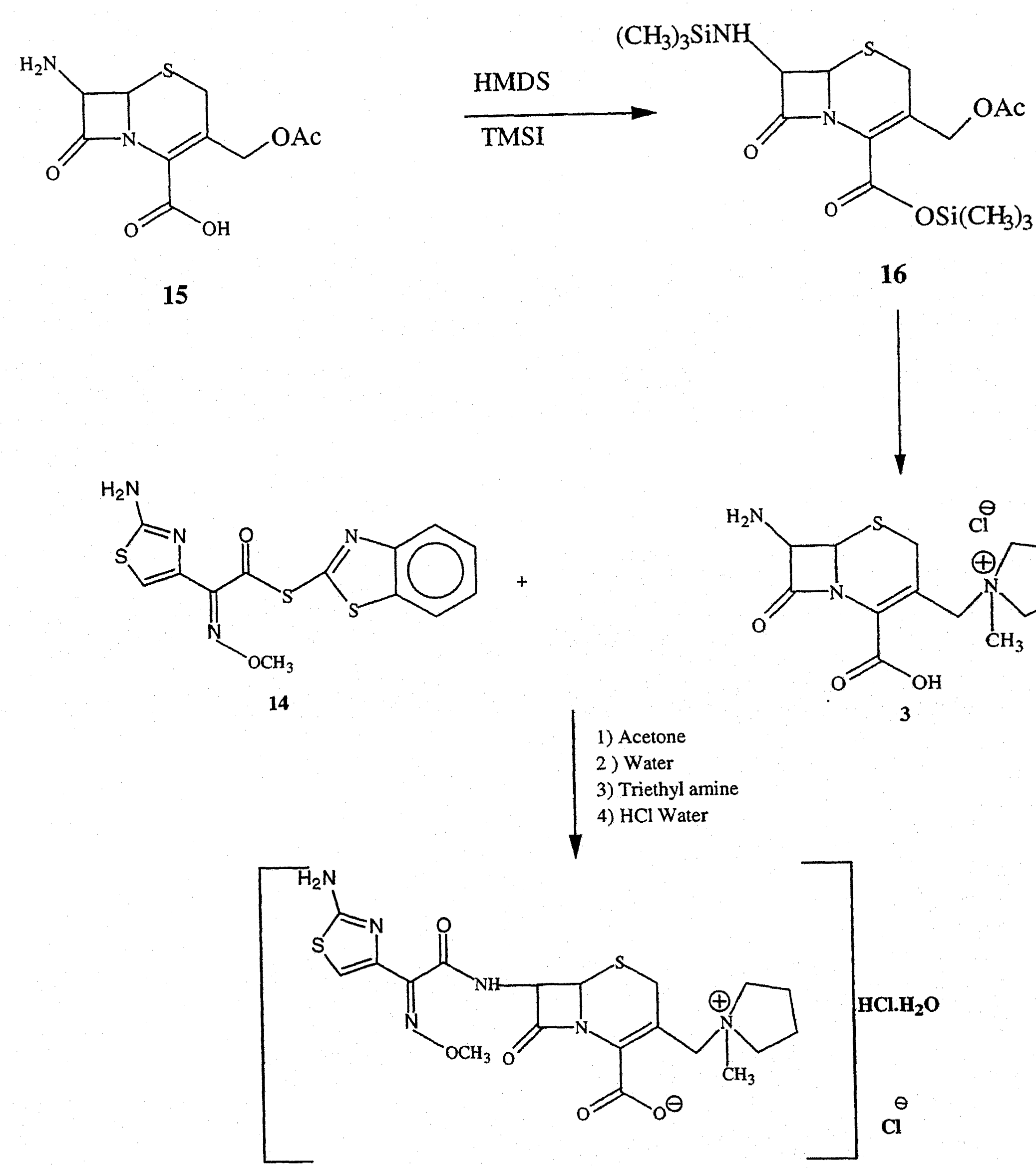
4.3 Present work

4.3.1 Selection of synthetic route

Selection of the synthetic scheme was made in parts from the available literature. Scheme **4.6** was used for preparation of 7-amino-3-[(1-methyl-1-pyrrolidino)-methyl]ceph-3-em-4-carboxylate. Acylation was done in accordance with scheme(**4.5**) and (Z)-(2-aminothiazol-4-yl)methoxy-iminoacetic acid-2-mercaptobenzothiazolylester was used for acylation (**5**). Schemes **4.1** and **4.2**

were not opted because of the long and cumbersome synthetic steps involved in it. Based on the commercial availability of the raw materials, 7-ACA was selected as the key starting material. The possible synthetic route for infringing cost effective process is as scheme **4.5.**

Scheme 4.6

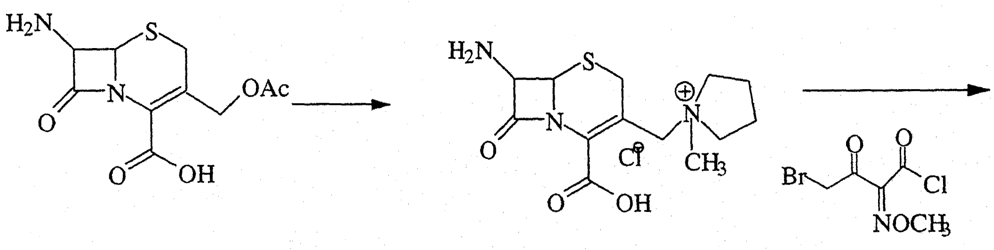


Cefepime hydrochloride
6

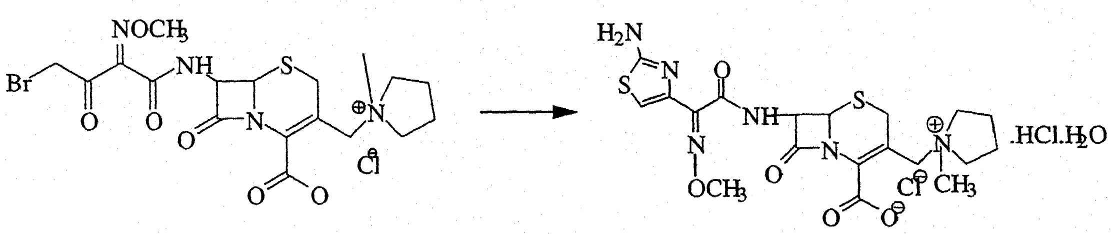
Similarly the proposed scheme (**4.7**) for non infringing and industrially feasible process is selected based on Ludescher J., Wolf S.[4] discloser and by taking 4-bromo- 2-methoxyimino-3-oxo-butyryl chloride instead of its chloro derivative and

reaction in aqueous media by avoiding silylating agent which required the anhydrous condition which is difficult to achieve industrially

Scheme 4.7



7-ACA 15 7-APC 3 17



BMPC 18 Cefepime 6

4.3.2 Preparation of 7-amino-3-[(1-methyl-1-pyrrolidino)-methyl]ceph-3-em-4-carboxylate (3) (7-APC)

Preparation of 7-APC (**3**) is performed in accordance with Gary M.F et. al [8] (as shown in scheme (3). 7-Amino Cephalosporanic acid is silylated with hexamethyl disilazane. The silylated 7-ACA is reacted with iodo trimethyl silane and N-methyl pyrrolidine complex, which on acidification with hydrochloric acid gives crude 7-APC. The crude product is purified in acetone water to get pure 7-APC. The details

of the process development and optimization of process parameters are described as below.

4.3.2.1 Selection of solvent

The preparation of (**3**) 7-APC from (**15**) 7-ACA gives different amount of Δ^2 isomer of 7-APC using different solvent. Minimum amount of Δ^2 isomer formed in 1,1,2-trichloro trifluoro ethane is reported by Donald G.et.al[7]. But (CFCs) are banned due to Ozone deplating chemicals. By performing the reaction in dichloromethane as a solvent gives 7-APC in 19 % yield. In further study, Silylation of 7-ACA is done in dichloromethane and reaction with iodotrimethyl silane-NMP complex in cyclohexane gives 7-APC in 36.7% yield. But the reaction operation is tedious as dichloromethane to be distilled completely otherwise results in Δ^2 isomer formation and may causing lower yield. When cyclohexane used as a solvent for Silylation and reaction affords 7-APC with 40.8% yield . Hence cyclohexane was the solvent of choice for the said reaction.

4.3.2.2 Purification of 7-APC (3)

In order to get the better purity and description of the 7-APC its purification from different solvents were attempted. The solvents attempted for crystallization were water-isopropyl alcohol water-acetone. Water-acetone mixture was found to be the most appropriate solvent system for purification in terms of yield and quality of the purified product. Isolation at different pH was studied like 1.0 , 3.0 pH , 5.0 pH preferred pH is 1.0 based on suitability in preparation of BMPC.

4.3.3 Preparation of Cefepime hydrochloride

4.3.3.1 Cefepime by using ammonium salt of 4-bromo- 2-methoxyimino-3-oxo-butyric acid.

There are two method for preparation of [1-[[(6R,7R)-7-[[(2Z)-(4-bromo-2-methoxyimino-3-oxo-butyryl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methyl-pyrrolidinium hydrochloride (**18**). like silyl route and aqueous route as described Ludescher J., Wolf S.[3]. Silyl route requires the anhydrous reaction condition use of silylating agent increases the manufacture cost hence aqueous route is preferred. 4-Bromo-2-methoxyimino-3-oxo-butyryl chloride was prepared from ammonium salt of 4-bromo-2-methoxyimino-3-oxo-butyric acid by phosphorous pentachloride in dichloromethane. The reaction is carried out in acetone-water. The details of the process development and optimization of process parameters are described as below.

4.3.3.2 Type of 7-APC used.

BMPC (**18**) was prepared from 7-APC (**15**) isolated at different pH. BMPC was prepared from 7-APC isolated at pH 5 gives 93.5% purity and 55.2% yield. From 7-APC isolated at 3 pH giving BMPC (**18**) in 77.3% purity. When 7-APC isolated at 1pH affords BMPC with 94.91% purity and 67 % yield. Thus 7-APC monohydrochloride dihydrate (isolated at 1 pH) is type of choice for preparation of BMPC.

4.3.3.3 Temperature of reaction

Condensation reaction at 0°C, -5° -10°C were carried out among these the best results obtained in terms of BMPC purity at –5°C.

4.3.3.4 Purification of BMPC

In order to get the better purity (minimum impurity) of the condensed intermediate (BMPC), its purification by acid base neutralization was attempted in acetone-water. The temperature attempted for purification were 5°C, -5°C and –10°C. The choice of temperature is –5 to –10°C for purification in terms of yield and quality of the purified product.

4.3.3.5 Preparation of Cefepime hydrochloride

BMPC (**18**) is neutralized with base. The resulting free base is treated with thiourea, which on acidification gives Cefepime hydrochloride. The details of the process development and optimization of process parameters are described as below.

4.3.3.6 Selection of base

Neutralization of the BMPC (**18**) was attempted with triethyl amine, n-methyl morpholine and aqueous potassium bicarbonate. Aqueous sodium carbonate. Aqueous potassium bicarbonate was selected as a base of choice with respect to purity obtained in process.

4.3.3.7 Purification of Cefepime hydrochloride (6)

Purification of Cefepime hydrochloride was done by dissolving it in DMW and precipitating by addition of acetone. Purification was preformed at 12°C and 28°C

precipitation at temperature 28°C gives better minimization of impurity. To meet the pharmacopial requirement it was again purified by dissolving in methanol and adding acetone in to it.

4.3.4 Preparation of Cefepime (6) by using (Z)-(2-aminothiazol-4-yl)methoxy-iminoacetic acid-2-mercaptobenzothiazolylester(14)

Preparation of Cefepime hydrochloride is performed in accordance with Ludescher J., Wolf S.[4] and Gong Ping et al.[5] as shown in scheme 3.5. 7-Amino-3-[(1-methyl-1-pyrrolidino)-methyl]ceph-3-em-4-carboxylate (7-APC)was treated with (Z)-(2-aminothiazol-4-yl)methoxy-iminoacetic acid-2-mercaptobenzothiazolylester (MAEM) in presence of Triethyl amine base in Acetone – Water .Final product was isolated as hydrochloride salt in same solvents by using hydrochloric acid .

Selection of solvents

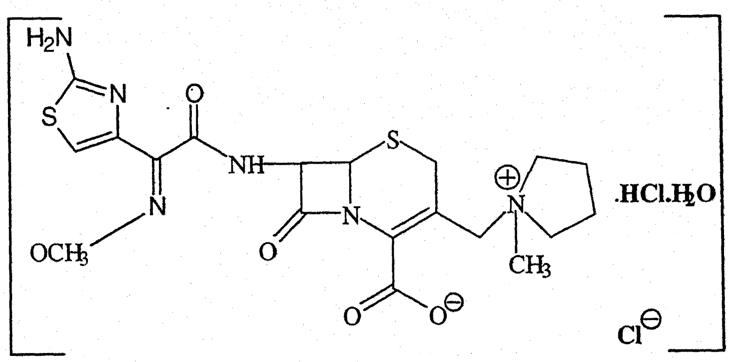
Preparation of Cefepime hydrochloride by using 7-APC and MAEM was tried in different aqueous solvents such as THF- DMAc, Acetone, Dichloromethane And DMF Water. Among these all DMF –Water is the choice of reaction media, which gives better quality, and yield of Cefepime hydrochloride

Reaction was tried in different quantity of DMF from 4 volumes to 7.64 volumes w.r.t. to 7-APC . Better yield and quality was obtained by using DMF 7.64 volumes. To meet the pharmacopial requirement dissolving in methanol and adding acetone in to it again purified it.

4.4.3 Synthesis, isolation and characterization of impurities:

US Pharmacopoeia specifies that the purity of Cefepime[9] should be between 90 to 105 %. However, most of the reported methods are associated with the formation of varying amounts of impurities and hence there is need to identify the cause of formation and characterization of impurities

Mainly two process impurities were observed in the Cefepime during its synthesis in the range of 0.2 to 1.5%, the molecular weight of these impurities were checked by LC-MS and from the molecular weight the structure of the impunities were illustrated as shown below structure (21) and (22). Efforts were made for the synthesis of these impurities.



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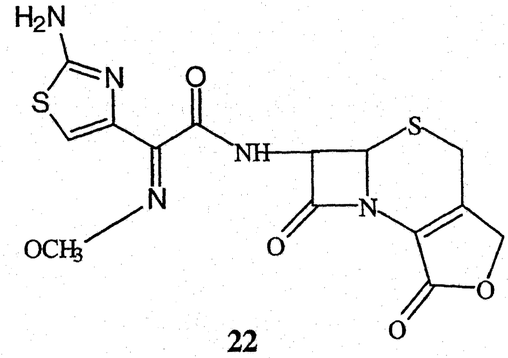
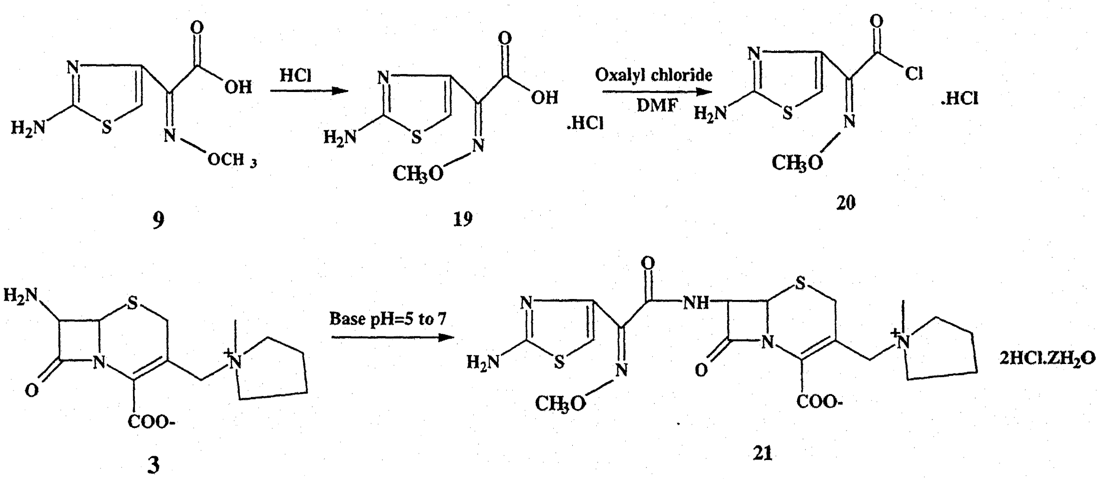


22

The impurity (**21**), which is anti isomer of Cefepime formed during the synthesis of Cefepime hydrochloride in the range of 0.5 to 1.5%. To characterize this impurity it was chemically synthesized as per displayed in the scheme-4.8

Scheme 4.8



Synthesis of the anti isomer of Cefepime (19) involved two steps in which acid chloride of 2-(Amino-4-thiazolyl)-2-(E) methoxy imino acetic acid (21) was reacted with 7-amino-3- [(1-methyl-1-pyrrolidino)-methyl] ceph-3-em-4-carboxylate (3) in aqueous acetone at pH 5.0 to 7.0. The resulting solution was acidified to pH 1.0 to 1.2 by hydrochloric acid and was precipitated by adding acetone to afforded anti isomer of Cefepime.The detailed procedure is given in experimental section.

The impurity (22) is the process impurity formed during the synthesis of Cefepime hydrochloride. This impurity generally formed in very strong acidic or basic condition (Jeffery et al 1961) it was synthesized by acidic treatment of Cefepime hydrocloride in 0.1 N hydrochloric acid at room temperature for 5-6 h. Reaction mixture was then extracted in dichloromethane. The organic layer was concentrated under reduced

pressure and the slowly added in di-isopropyl ether to give the impure product containing impurity (22). The impure compound was then purified by dissolving in dichloromethane and crystallization with di-isopropyl ether to afforded the impurity (22) in pure form.

4.5 Conclusion

This work provides the process for preparation of the pure and stable, operationally simple and commercially viable and utilizes the solvent aqueous media and avoids the use of silylated reagents where monitoring silylation reaction is difficult.

Similarly it provide the low costing process by involving minimum steps to give pure stable Cefepime which meets the pharmocopial and market requirement.

4.6 Experimental

7-amino-3-[(1-methyl-1-pyrrolidino)-methyl]ceph-3-em-4-carboxylate (3) (7-APC)

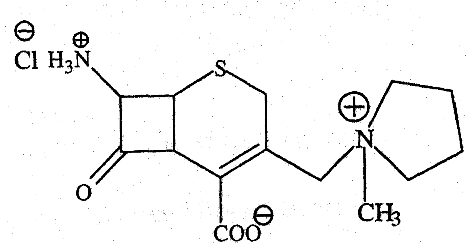
7-ACA (25 g, 0.0919 mole) was added to cyclohexane (200 ml) at 25° to 30°C followed trimethyliodosilane (1.0 g, 0.005 mole) and hexamethyldisilazane (17.77 g, 0.110 mole). The reaction mass was heated to reflux temperature and refluxed for 2 hours under nitrogen purging and cooled to 10°C. The reaction mass stirred for 2 hours to obtain silyated 7–amino-3-(cepholosporonic acid)7-ACA)compound (**16**)

To this solution N-methyl pyrrolidine (10.95 g, 00268 mole) was added and the reaction mixture is stirred at 34 to 36°C for 36 hours and monitored by HPLC till

quantitative conversion to the silylated 7-ACA is achieved. Then the reaction mass was cooled to 5-10°C and the methanol (12.5 ml) was added to resulting reaction mass followed by addition of 3 N hydrochloric acid (75 ml) at 5° to 10°C. The reaction mass is stirred for 30 minutes and the layers are separated. The aqueous layer is diluted with acetone (150 ml) followed by the addition of 2.5 g of activated carbon and sodium dithionite (0.25 g) to remove the colored solid impurities. The reaction temperature is maintained at 10° to15°C. The carbon was filtered and filtrate was basified to pH 1 t0 1.2 by ammonia solution. The acetone (500 ml) was added to the mixture at 10-15 °C and slurry was cooled to 5°. The product is filtered and washed with acetone (125 ml) to give Crude 7-APC ((**3**) in the form of pale yellow powder.

Purification of 7-amino-3-[(1-methyl-1-pyrrolidino)-methyl]ceph-3-em-4-carboxylate (3) (7-APC)

Crude 7-APC (35g) was added to water (88 ml) at 25°C and stirred for 15 min . Hydrochloric acid (17%) was added to it till clear solution obtained . To this clear solution added carbon (5 g) and stirred for 30 min 22-25°C. Carbon was filtered was added . The slurry was stirred for 2 h at 0-5°C and filtered ,washed with acetone (125 ml) to afforded the pure white powder of 7-APC (**3**).



(3)

^{1}H NMR Spectrum (DMSO- d_6, δ in ppm):

2.08 envelop,(4 H, -N(CH_3)$\underline{CH_2CH_2}$-), 2.49 s (3H, -$\underline{CH_3}$N),3.53-3.55, m (5H,-N(CH_3) $\underline{CH_2CH_2}$; S$\underline{CH_2}$),3.89,d (1H-S$\underline{CH_2}$),4.09 d (1H, =CCH2N-),4.64 d (1H,-COCH$\underline{CH}$S-), 5.15 d (1H, - CO$\underline{CH}$CHS-),

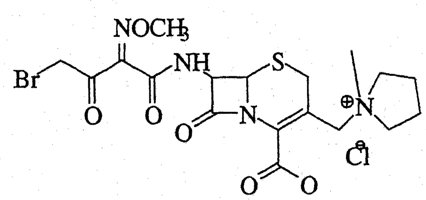
7-[4-Bromo-2(Z)-methoxyimino-3-oxobutyramide]- 3-[(1- methyl -1-pyrolidino) methyl] ceph-3-em-4-carboxylate

A mixture of phosphorous pentachloride (7.45 g, 0.0357 mol.), dichloromethane (50ml) was cooled to –10°C. Ammonium salt of 4-bromo-2-oxyimino-3-oxo butyric acid (8.67 g ,0.036 mole) was added in lots at –10 to-15°C. Mixture was stirred at 30°C for 30 min. and 0 to 5°C for 30 min. Dichloromethane was evaporated completely. To this acetone (25 ml) was added. In another flask the mixture of acetone (50 ml), water (50 ml) and 7-amino-3-[(1- methyl1-pyrolidino) methyl] ceph-3-em-4-carboxylate hydrochloride (10g) was cooled to –10°C. pH was adjusted to 6.5 by 25% potassium bicarbonate solution. Acid chloride was added slowly by maintaining pH 5.4 to 5.8 at –5 to –10°C. Mixture was stirred for 20 minute. pH was adjusted to 0.7 slowly by concentrated hydrochloric acid at 0 to –5°C. Mixture was stirred for 2 hr at same temperature, filtered washed with (1:1) acetone-water (40ml) and acetone (40ml). Drying under vacuum at 40°C affords 7-[4-Bromo-2(Z)-

methoxyimino-3-oxobutyramide]- 3-[(1- methyl -1-pyrolidino) methyl] ceph-3-em-4-carboxylate hydrochloride (12 g, 74.4%).

Purification of 7-[4-bromo-2(Z)-methoxyimino-3-oxobutyramide]- 3-[(1- methyl -1-pyrolidino) methyl] ceph-3-em-4-carboxylate hydrochloride

The mixture of acetone (50 ml), water (50 ml) and 7-[4-Bromo-2(Z)-methoxyimino-3-oxobutyramide]- 3-[(1- methyl -1-pyrolidino) methyl] ceph-3-em-4-carboxylate hydrochloride (12 g) was cooled to –10°C. pH of the mixture was adjusted to 4 to dissolve completely by 25% potassium bicarbonate. Carbon (10 g) was added and mixture was stirred for 30 minutes at 0 to 5°C. Filtered and the pH of filtrate was adjusted by dilute hydrochloric acid to 0.7. Slurry was stirred for 2 hr at 0 to-5°C, filtered washed with water and acetone, drying under vacuum at 40°C affords (10 g) pure 7-[4-bromo-2(Z)-methoxyimino-3-oxobutyramide]- 3-[(1- methyl -1-pyrolidino) methyl] ceph-3-em-4-carboxylate hydrochloride



18

^{1}H NMR Spectrum (DMSO- d_6, δ in ppm):

2.06 s (4 H, -CH_3-N-$(CH_2)_2$<u>$(CH_2)_2$</u> 2.89 s (3H, -N-CH_3), 3.36-3.44 m (5H, pyrrolidinyl-H,-<u>SCH_2</u>) , 3.92 S (1H,-S<u>CH2</u>), 4.03 s (3H, <u>OCH3</u>), 4.14-4.63 d (2H, -<u>CH2</u>-N-) ,5.25-5.27 d (1H,-COCH<u>CH</u>S-), 5.71 d (1H, - CO<u>CH</u>CHS-),

9.48-9.52 d (1H, -<u>NH</u>-).

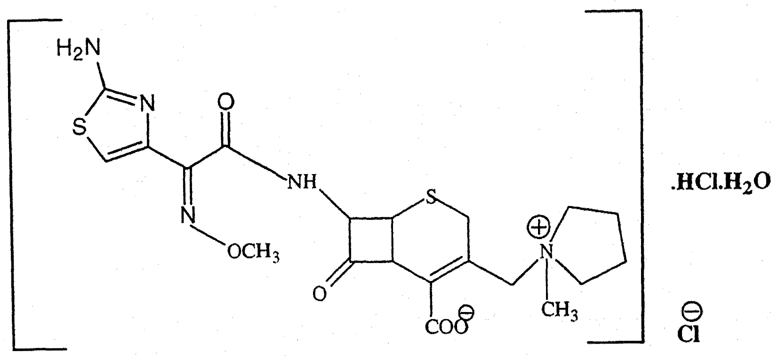
I R (KBr) Cm^{-1}: 2343-3172,1782,1707,1680,1606,1544,1421.

Preparation of 7-[2-(2-aminotiazol-4yl)-2-(z)-methoxy-iminoacetamido]-3-[(1-methyl-1-pyrolidino)-methyl] ceph-3-em-4carboxylate (cefepime).

A mixture of 7-[4-bromo-2(Z)-methoxyimino-3-oxobutyramide]- 3-[(1- methyl -1-pyrolidino) methyl] ceph-3-em-4-carboxylate hydrochloride (8 g, 0.01481mol), acetone (40ml), water (40ml) was cooled to 10°C. pH of mixture was adjusted to 4 by 25% potassium bicarbonate , thiourea (1.568g, 0.02063 mol) was added. Mixture was stirred for 90 min at pH 4.2 to4.5 at 25°C. Carbon (1.2 g) was added and stirred for 1 hr, filtered washed with water (8 ml). pH of filtrate was adjusted to 0.5 by concentrated hydrochloric acid at 8-10°C. Acetone (340 ml) was added slowly in 2 hr at 8-10°C. After stirring for 1 hr at 8-10°C mixture was filtered, washed with acetone (40 ml) gives Cefepime 8.8 g (94.5% yield, purity 99.3%).

Purification of Cefepime:

Cefepime (8) g was dissolved in DM water (40 ml) under stirring. To that acetone (120 ml) was added slowly in 30 min at 25-28°C. Mixture was stirred for 30 min at same temp. Acetone (200 ml) was added slowly in 1hr at 25-28°C. Mixture was stirred for 1hr, filtered and washed with acetone (40ml), dried under vacuum at 40°C gives Cefepime 7.5 g (93% yield, purity 99.7%).



6

^{1}H NMR Spectrum (D_2O, δ in ppm):

2.09 s (4 H, $-CH_3$-N- $(CH_2)_2\underline{(CH_2)}_2$, 2.88 s (3H, -N-$\underline{CH_3}$), 3.3 –3.6 m (5H, pyrrolidinyl-H,-$\underline{SCH_2}$), 3.85 d (1H,-S$\underline{CH2}$), 3.94 s (3H, $\underline{OCH3}$), 4.58-4.69 d (2H, -$\underline{CH2}$-N-) ,5.23-5.25 d (1H,-COCH$\underline{CH}$S-), 5.71 d (1H, - CO$\underline{CH}$CHS-),

7.02 s (1H, S-$\underline{CH}$=).

I R (KBr) Cm^{-1}: 2814-3230,1772,1728,17051654,1635,1587,1568,1544,1446.

Preparation of 7-[2-(2-aminotiazol-4yl)-2-(z)-methoxy-iminoacetamido]-3-[(1-methyl-1-pyrolidino)-methyl] ceph-3-em-4carboxylate (Cefepime) by using (Z)-(2-aminothiazol-4-yl) methoxy-iminoacetic acid-2-mercaptobenzothiazolylester

To a mixture of water (40 ml) and N,N-Dimethyl formamide (66 ml) added 7- APC **3**, 10 g .0.03 mole) at 25°C and cooled to 5°C. The pH of the mixture was adjusted to 6.5 to 7.0 with trimethyl amine (3.3 g, 0.033 mole) in 1 h under stirring at about 0-5°C to get clear solution then Z-(2-aminothiazol-4-yl)methoxy-iminoacetic acid-2-mercaptobenzothiazolylester (12.12 g, 0.346 mole)was added . The reaction mixture was stirred for 3.0 h with maintaining the

pH 6.5 to7.0 by trimethyl amine . The reaction was monitored by HPLC till qualitative conversion of 7-APC TO Cefepime product is achieved. Dichloromethane (100 ml) was added to the mixture and stirred for 15 min , water solution was separated and charcoalised for 15 min. Acetone (400 ml) added slowly to the clear filtrate in 90 min at 10 to 12 °C and cooled to 0°C. The solid was filtered ,washed with acetone (50 ml) and dried under vacuum to furnished the pure white crystalline product (**6**) 12 g

Preparation of 7-[2-(2-aminotiazol-4yl)-2-(E)-methoxy-iminoacetamido]-3-[(1-methyl-1-pyrolidino)-methyl] ceph-3-em-4carboxylate (Cefepime-E-isomer).

Step A

The solid 2-(Amino-4-thiazolyl)-2-(z) methoxy imino acetic acid (25 g, 0.124mole) was suspended in tetrahydrofuran (200 ml & to this suspension dry Hydrochloric acid gas was bubbled for about 120 min. The resulting solution was stirred for 15 hour at 20-25°C Then the product was filtered, washed with tetrahydrofuran (100 ml). Dried the product under vacuum at 50°C for 8 hr to furnish 20 g of 2-(Amino-4-thiazolyl)-2-(E) methoxy imino acetic acid. The confirmation of isomerisation monitored by HPLC under same condition.

Mobile phase: (A) 0.05 M ammonium acetate: Methanol (95:5)

pH by 4.2 by H_3PO_4

(B) 0.05 M ammonium acetate: Methanol (50:50)

pH by 3.75 by H_3PO_4

Column: Inertsil ODS 3v 250X4.6 mm, 5µ

Flow: 1.5 ml/min at wavelength 254 nm

Oven temperature: 40 °C.

The retention time of syn-isomer appeared at 2.46 while anti-isomer appeared at 7.1. This anti-isomer used for the preparation of the title compound.

Methylene chloride (100 ml) is cooled to 20-25°C, and 2-(Amino-4-thiazolyl)-2-(E) methoxy imino acetic acid (**20**, 5.44g 0.0314 moles) is added under stirring. The resulting solution is cooled to –25° to —30°C and Phosphorous pentachloride (7.09 g 0.0340 moles) was added to it. The mixture was agitated at 0 to 5° C for 45 minutes under nitrogen gas purging for removal of hydrochloride gas generated during acid chloride formation. Dichloromethane was distilled under reduced pressure and acetone (15 ml) was added to the residue. The reaction mixture was cooled to –15 °C.

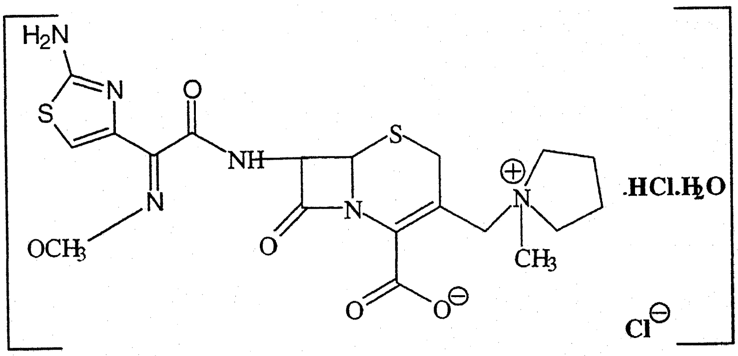
Step B

7-APC [(**3**), 10 gm 0.0299 moles] was suspended in acetone (50 ml) at 25° to 30°C followed by addition water (50 ml). The reaction mass was cooled to –5°C and basified to pH 5.3 to 7 by using 25% potassium bicarbonate solution in 45-60 min to obtained the clear solution of potassium salt of 7-APC.

<u>Step C</u>

To a solution of acid chloride of 2-(Amino-4-thiazolyl)-2-(E) methoxy imino acetic acid (**20**) above prepared potassium salt of 7-APC , was added with maintaining the, pH at 5.2 to 5.8 by using 25% potassium bicarbonate solution. The reaction mixture was stirred at –5° to –7°C and monitored by HPLC till quantitative conversion to the condensed product is achieved. The reaction time was about 20

min hours. The resulting reaction mass was acidified to pH 1.5 to 1.8 by hydrochloric acid. To the reaction solution acetone (400 ml) was added in 2 h at 28 to 30°C.The slurry obtained is stirred for 1 hour at 28-30° and filtered off. The solid product was washed with acetone (50 ml) and dried under vacuum to afforded E-isomer of the Cefepime (**21**) 10 g.



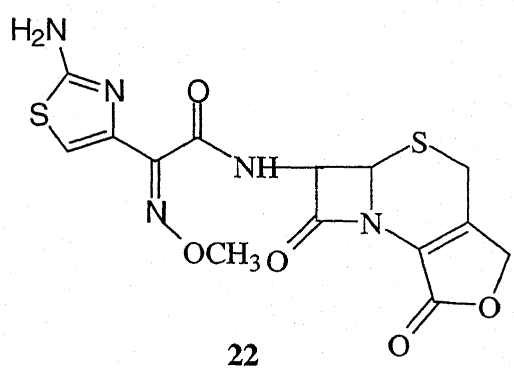
21

[1]H NMR Spectrum (DMSO- d_6, δ in ppm):

2.05 s (4 H, -CH_3-N-$(CH_2)_2$$\underline{(CH_2)_2}$,2.92 s (3H, -N-$\underline{CH_3}$), 3.43 –3.6 8m (5H, pyrrolidinyl-H,-$\underline{SCH_2}$), 3.85 d (1H,-S$\underline{CH2}$), 3.96 m (3H, $\underline{OCH3}$), 4.26-4.61 d (2H, -$\underline{CH2}$-N-) ,5.29-5.31 d (1H,-COCH$\underline{CH}$S-), 5.79-5.86 q (1H, - CO$\underline{CH}$CHS-), 7.54 M (1H, S-$\underline{CH}$=), 9.51-9.55 d (2H , -N=C-$\underline{NH2}$)

Preparation of N-(2R)-1,7-dioxo (5-hydro-2H, 4H,2aH-azetidinol [2,1-b] furano [3,4-d] 1,3-thiazan-2-yl)) (2 Z) –2-(2-amino (1,3 –thiazol –4- yl)) –3-aza-3-methoxyprop-2-enamide (Lactone impurity):

To a solution of 7-[2-(2-aminotiazol-4yl)-2-(z)-methoxy-iminoacetamido]-3-[(1-methyl-1-pyrolidino)-methyl] ceph-3-em-4carboxylate (25 g, 0.0437 mole) in 0.1 N hydrochloric acid (150 ml) was stirred foe 6 h at 25-27°C. The reaction mixture was extracted in dichloromethane (200 ml) and organic solution was concentrated under reduced pressure. Di-isopropyl ether (250 ml) was added slowly to the concentrated reaction mass and the slurry was stirred for 1 h at 5-10°C. The solid was filtered, washed with Di-isopropyl ether (50 ml). The crude product obtained was purified by dissolving in dichloromethane (50 ml) and crystallized by adding Di-isopropyl ether (250 ml) slowly in 2 h. The slurry was stirred for 1 h at 5-10°C and filtered, washed with Di-isopropyl ether (50 ml) to furnished pure compound (22) 2.5 g .



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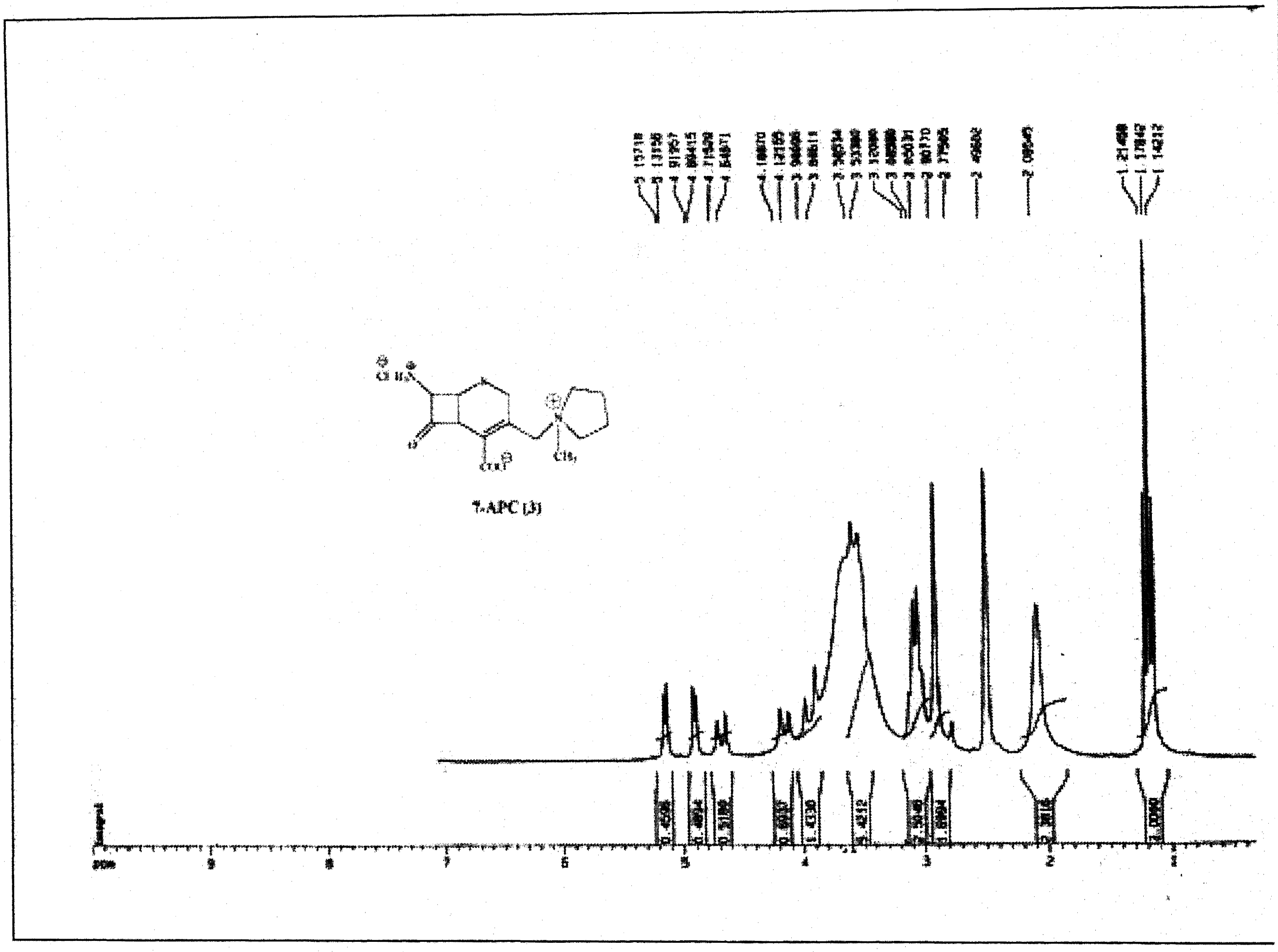
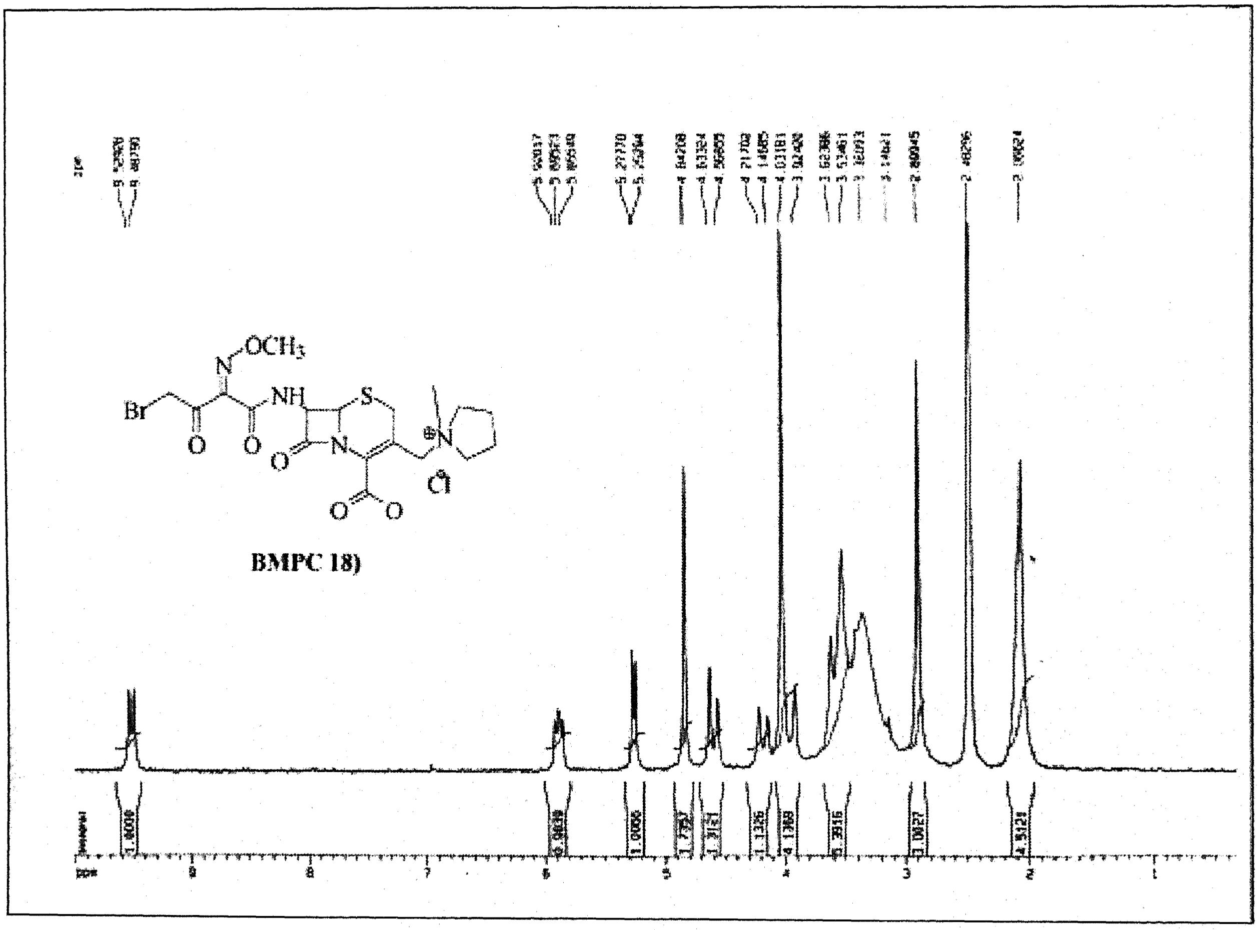
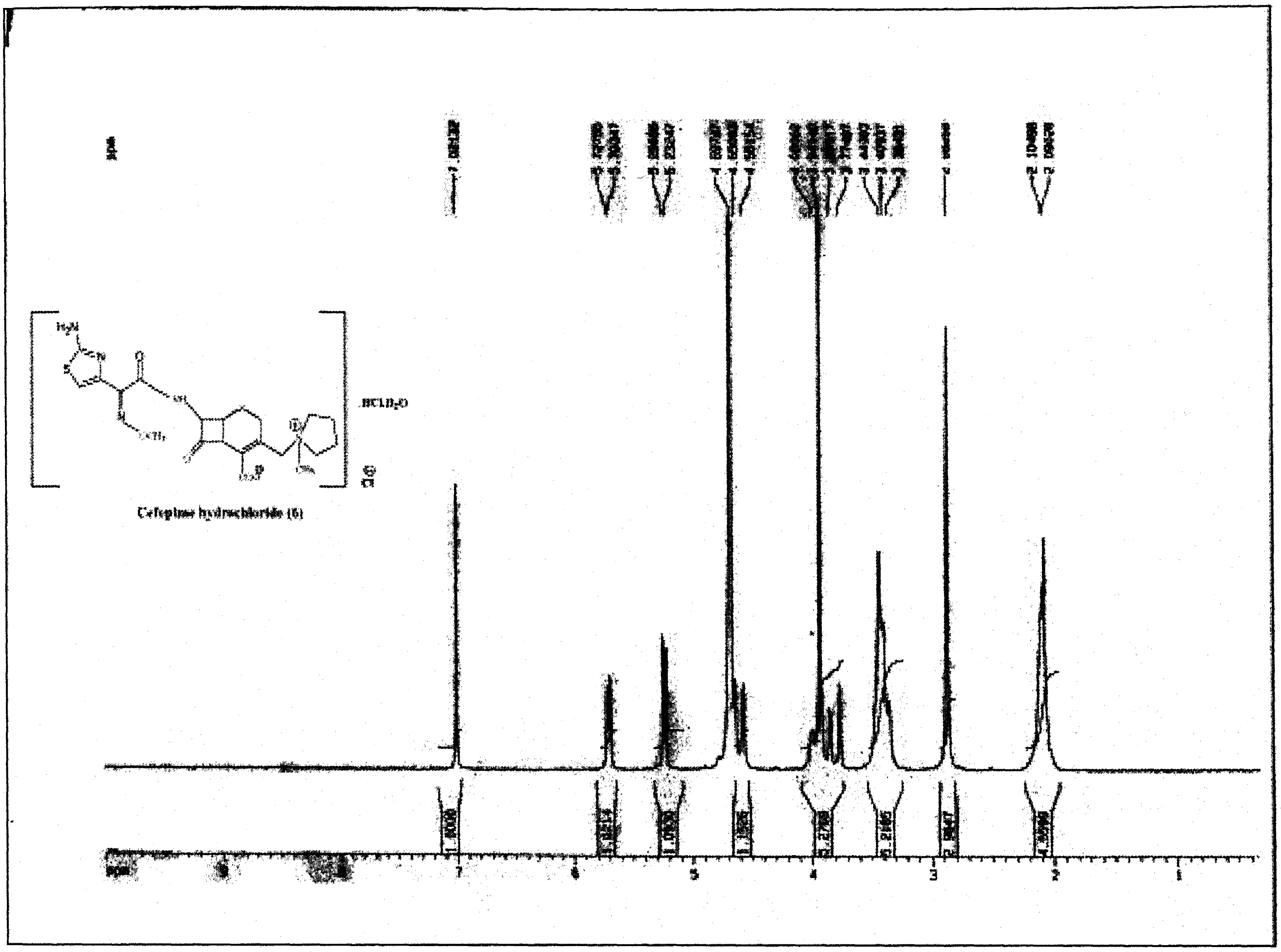
^{1}H NMR Spectrum (DMSO- d_6, δ in ppm):

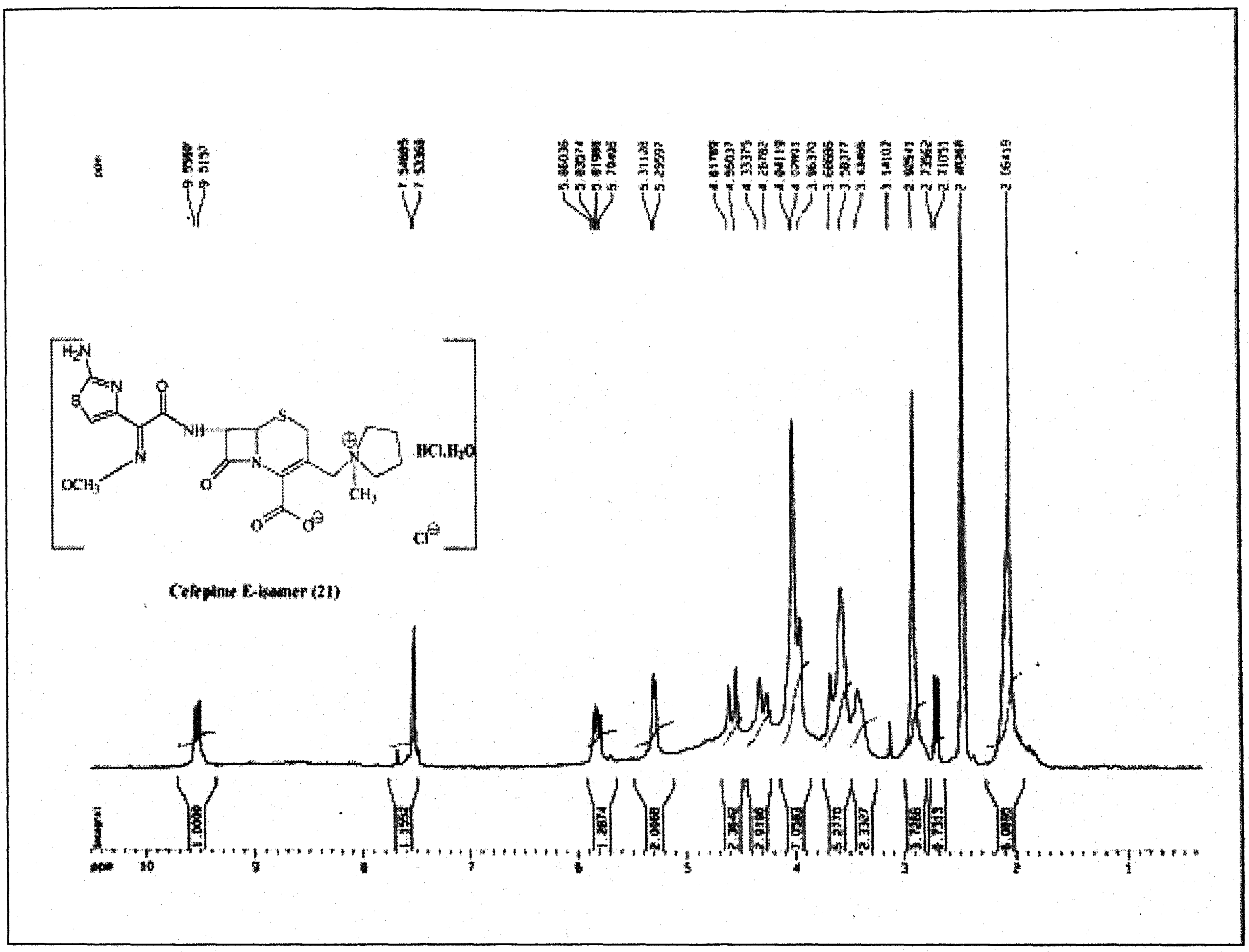
3.81 s (2H, - S(CH2-),3.93 s (3H, -N-OCH3) ,5.05 s (2 H, -CH2-O-C=O), 5.19 d (1H,-COCHCHS-), 5.5.96 d (1H, - COCHCHS-), 6.88 s (1H , -S-CH=) 9.87 d (1H, -NH-).

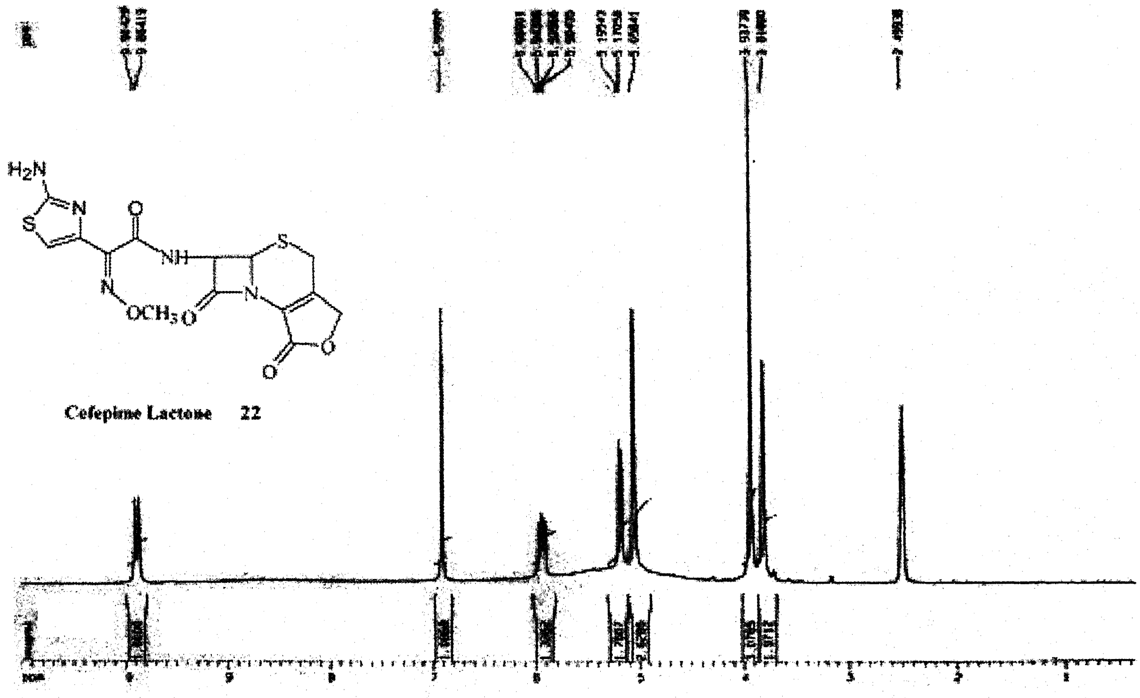




BMPC 18)




HCl.H2O
Cefepime E-isomer (21)



Cefepime Lactone 22

4.7 References

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2 Takayuki Naaito, Shimpei Abukari, Kamachi H., Yukio N., Jun Okumara; J. Of Antibiotics **10**,1395-1107 (1986).

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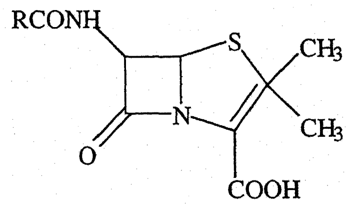
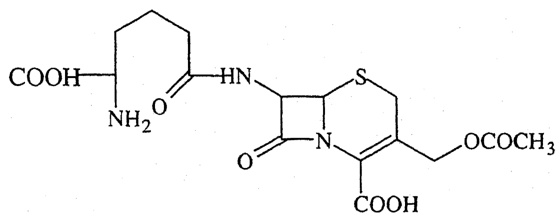
Summary

Chapter I of the thesis includes the brief introduction of the cephalosporin antibiotic. It is over seventy years since Alexander Fleming observed antibiotics between a Penicillium mould and bacterial cultures and gave the name penicillin to the active principle. Although it was proposed in 1943 that penicillin (1) contained a β –lactam ring, this was not generally accepted until an X-ray crystallographic determination of the structure had been completed.

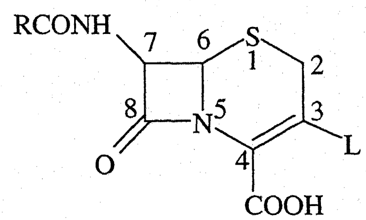


(1)

Penicillin was the first naturally occurring antibiotic to be characterized and used in clinical medicine. It is now seen as the progenitor of the β-lactam family of antibiotics, which are characterized by the possession of the four membered β-lactam ring. Penicillins (**1**) and Cephalosporin (**2**) the second member of β-lactam antibiotics family were both originally discovered in fungi but later detected in streptomycetes. The basic molecule of the cephalosporin was generated from Cephalosporin –C.


Cephalosporin C

The classification of the cephalosporins are depends on the activity of the molecule towards gram –ve and gram +ve bacteria. The activity differs molecule to molecule by changing substitution at C-7 and C-3 position of the cephalosporin ring (2). Based on the different activity cephalosporins are classified in to first, second, third and fourth generation.


(2)

Chapter II of the thesis includes the work carried for the development of cost effective process of the Cefixime trihydrate cephalosporin antibiotic, the work also provides for identification, synthesis and characterization of the impurities formed during its synthesis.

Cefixime is the first member of third generation orally active Cephalosporin· These third generation Cephalosporins are distinct from the older β-lactam antibiotics in their intensive antibacterial activity against the wide range of gram-negative bacteria. The exceptional antibacterial activity of the third generation

Cephalosporins has been shown to be based on both their enhanced affinity for the target enzymes and their high stability to β lactamase.

Because of its therapeutic usefulness and efficient broad spectrum of activity, it is required to prepare the product with high purity and yield, with minimum level of impurities, coupled with ease of operation and, more importantly, with low production cost.

Selection of the synthetic scheme was made in parts from the available literature based on the cheaper and commercially available raw materials.

Reaction of GCLE(**22**), in presence of sodium halide and triphenyl phosphine in the mixture of Dimethyl formamide and methylene chloride yielded the corresponding phosphonium salts which on reaction with formaldehyde in presence of sodium carbonate furnished 4-p-methoxybenzyl-3-vinyl-7-phenyl-acetamidocephem carboxylate hereinafter referred as 3-VBA(**24**)

p-Toluenesulphonate salt of p-methoxyphenyl-7-amino-3-vinylcephem-4-carboxylate namely 7-Amino Ester salt (**25B**), was prepared by the reaction of p-methoxybenzyl-7-phenylacetamido-3-vinyl cephem-4-carboxylate(**24**) with base and PCl_5 to give iminochloride which on treatment with methanol resulted in the hydrochloride salt of p-methoxybenzyl-7-amino-3-vinylcephem-4-carboxylate(**25**) which is very unstable. This, on treatment with ammonia followed by treatment with p-toluenesulphonic acid in ethyl acetate resulted in the p-toluenesulphonate salt of p-methoxybenzyl-7-amino-3-vinylcephem-4-carboxylate (**25B**)in stable form (7-Amino Ester Salt).

Since the isolated hydrochloride salt of p-methoxybenzyl-7-amino-3-vinylcephem carboxylate was unstable and picking up the colour, p-TSA salt of 7-Amino Ester was prepared after making freebase of 7-Amino Ester. Hydrochloric acid salt with ammonia, followed by treatment with p-Toluene sulphonic acid .

Cefixime is prepared from p-Toluenesulphonate salt of p-methoxyphenyl-7-amino-3-vinylcephem-4-carboxylate (**25B**) by condensation with DATMA (21) to give Cefixime ester which on *in-situ* deprotection to afford Cefixime,

To get the stable and pure morph of Cefixime Trihydrate was purified in ethanol water mixture (1:2) ratio.

Identification, synthesis and characterization of the Cefixime impurities.

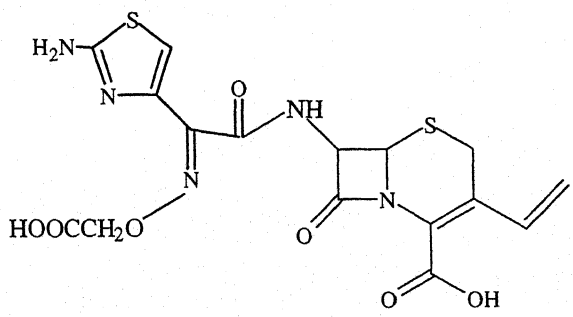
Cefixime is pharmaceutical product, to meet the quality requirement of pharmacopoeia in terms of minimum impurities which are mentioned in European pharmacopoeia we have directed our research efforts towards making a process for the preparation of Cefixime which overcome not only the drawbacks of the reported methods but which is operationally simple, easily producible on an industrial scale and which give Cefixime Trihydrate having good quality, stability, solubility and impurity profile.

Cefixime is pharmaceutical product, to meet the quality requirement of pharmacopoeia in terms of minimum impurities, which are mentioned in European pharmacopoeia. The structure of impurities namely D, E, F.and I are given in the European pharmacopoeia the same impurities tried to synthesized in the present work

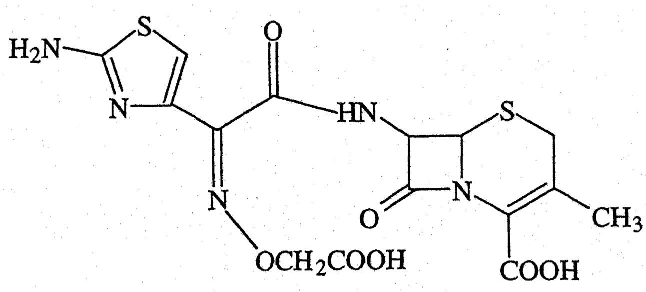
Beside these known impurities one more impurity observed at RRT (2.08) in our product in the range of 0.05 to 0.4. The mass of the impurity checked by LC mass (680.66), from the mass the structure of impurity was predicted and

here-in referred as impurity LF. In the present work impurity LF was synthesized and characterized by NMR also confirmed by LC mass.

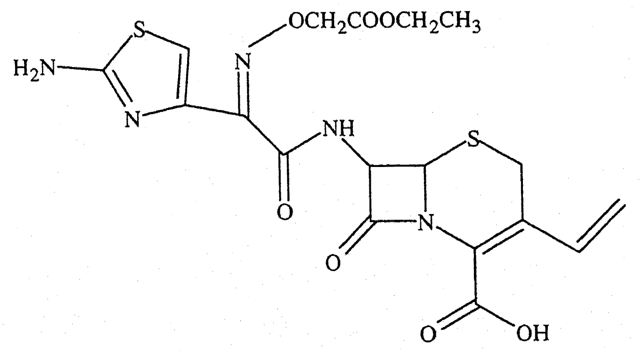
The structures of the pharmacopoeial (D, E, F, and I) and nonpharmacopoeial (LF) impurities are given below.



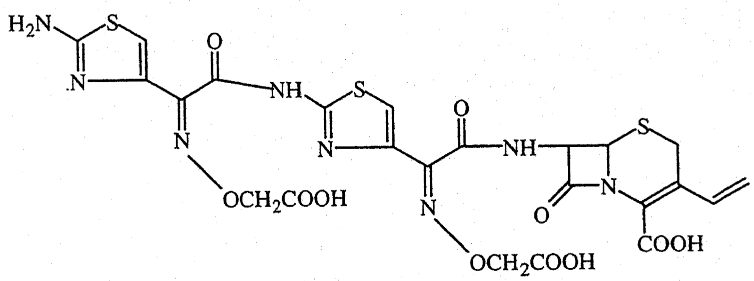
Impurity -D 42



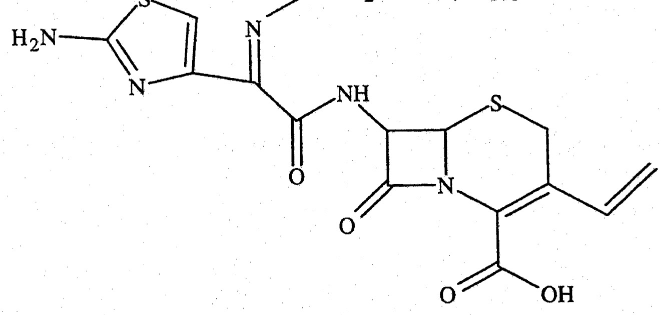
Impurity -E (47)



Impurity -F 50



Impurity LF 53



Impurity I (54)

Cefprozil monohydrate

The third chapter of the thesis includes the work for development of cost effective process of the Cefprozil monohydrate and synthesized its impurities which are formed during the preparation of Cefprozil monohydrate .

Cefprozil monohydrate is a semi-synthetic broad-spectrum Cephalosporin antibiotic consisting of 90:10 *Z/E* isomeric mixture. Cefprozil is an acid-resistant Cephalosporin due to the *p* - hydroxyphenyl-glycyl substituent at the 7 position. It acts by binding with target protein on the cell wall of susceptible bacteria, leading to inhibition of cell wall synthesis & the death of the cell. Its in vitro spectrum of activity is similar to those of known Cephalosporins Cefaclor & Cefuroxime axetil, and it is additionally active in vitro against penicillin-resistant strains of Streptococcus pneumoniae and certain anaerobes including Clostridium dificille. Cefprozil monohydrate was discovered and developed by Bristol-Myers.

Cefprozil is a commercially valuable and therapeutically useful oral Cephalosporin antibiotic effective in controlling diseases caused by a wide variety of Gram positive and Gram negative microorganisms.

Based on drawbacks of the reported methods for the synthesis of the Cefprozil we proposed the efficient and cost effective industrially viable process.

Preparation of 4-methoxybenzyl 7-phenylacetamido-3-[(Z/E)-propenyl]-3-cephem-4-carboxylate.

The present work provided the effective method for introduction of the alkenyl group at C3 position by using less expensive sodium bromide, carrying out the Wittig reaction in one-pot synthesis improved the yield and purity of the Wittig product.

Use of sodium chloride solution (20%) in the Wittig reaction played important role to control the impurity formation and to improve the yield and quality of the product.

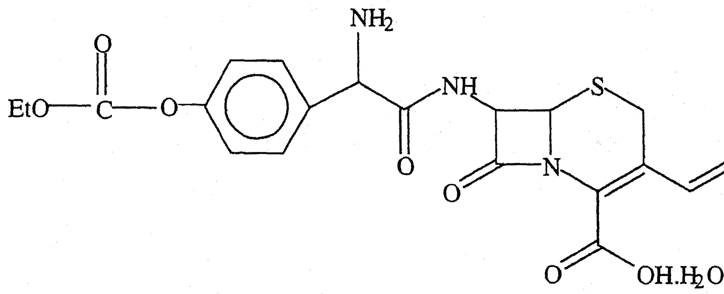
Preparation of 7-amino-3-[(Z/E)-propenyl]-3-cephem-4-carboxylic acid

p-Methoxybenzyl-7-amino-3-Propenyl cephem-4-carboxylate, namely 7-APCA ester was prepared by the reaction of p-methoxybenzyl-7-phenylacetamido-3-propenyl cephem-4-carboxylate with base and PCl_5 to give iminochloride which on treatment with methanol resulted the formation of p-methoxybenzyl-7-amino-3-propeny cephem-4-carboxylate . Since the p-methoxybenzyl-7-amino-3-propenylcephem carboxylate was not isolated, it was directly proceeded to deprotection by in-situ way by treatment with trifluoroacetic acid followed by precipitation with sodium hydroxide in D.M. Water resulted in the formation of 7- Amino 3-propenyl Cephalosporanic acid

All these improvements in conjunction have the advantage in providing Cefprozil monohydrate of high quality and in high yields, which moreover is convenient and cost effective.

Preparation of 7-[D-α-amino-α-(4-hydroxyphenyl) acetamido]-3-(1-propen-1-yl)-3-cephem-4-carboxylic acid

In the literature reported methods, wherein the sequence of addition is such that the Dane salt and the acylating agent are added first, the free acylating agent tends to react with the hydroxy group and leads to the formation of impurity (**26**) in higher quantities.



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Hence there is a need to have protected form of 7-APCA, which will activate the amino group in the 7-position, efficiently protect the carboxylic acid group, which will not require additional deprotection steps and can be deprotected in-situ during reaction work-up.

Therefore, we have developed here the simple and cost-effective method for the preparation of Cefprozil in high purity and yield.

In the present work we have found that the mixed carboxylic acid anhydride of a N-substituted--amino-p-hydroxy phenyl acetic acid or its salt (Dane salt,) can be prepared by a careful selection of a specific sequence and temperature for addition of the reagents so that it would be resulted in minimization of impurities during product formation. Mode of addition of dimethyl formamide plays important role in the preparation of mixed anhydride to control the carbonate impurity (**26**). Addition of dimethyl formamide before Dane salt at temp 20-25°C reduces the carbonate impurity where as addition of dimethyl formamide after (–50 to –55°C) Dane salt enhance

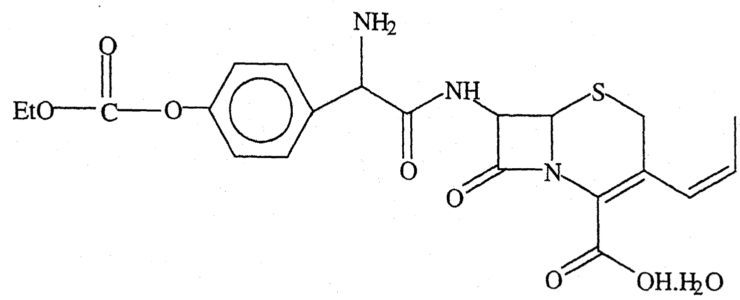
In the present work, the preferred sequence of addition is such that the acylating agent and base are mixed first so that they form a complex. Dane salt is then added

so that the acylating agent-base complex reacts preferentially with the carboxylic acid group and very little amount of the free acylating agent is available for reaction with the hydroxyl group and hence results in reduced quantities of impurity (**26**) as well as other impurities.

Synthesis, isolation and characterization of impurities:

Regulatory authorities all over the world are becoming very stringent about the purity of an approved drug. Especially there is growing concern about the nature and level of impurities present in such molecules. US Pharmacopoeia specifies that the purity of Cefprozil should be between 90 to 105 %. However, most of the reported methods are associated with the formation of varying amounts of impurities and hence there is need to identify the cause of formation and characterization of impurities

Mainly two process impurities were observed in the Cefprozil during its synthesis in the range of 0.2 to 1.5% , the molecular weight of these impurities were found by LC-MS and from the molecular weight the structure of the impunities were illustrated as shown below structure Carbonate impurity and N-glycil impurity . Efforts were made for the synthesis of these impurities and characterization by LC mass and NMR.



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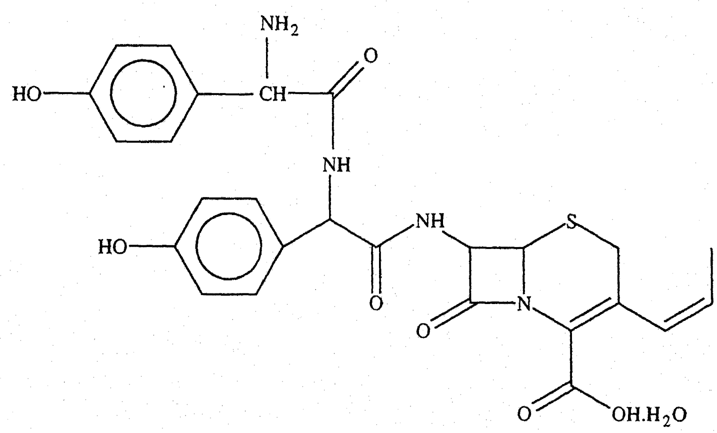

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The compound (**26**) was synthesized from 7-amino-3- [(Z/E)-propenyl]-3-cephem-4-carboxylic acid and N-substituted-α-amino-p-hydroxy phenyl acetic acid or it's salt (Dane salt) by using excess quantity of ethylchloroformate and N-methyl morpholine .as process described in experimental section of the thesis. The compound (26) was identified by LC mass and characterized by NMR.

Similarly the second N-glycil impurity was synthesized from silyl protected Cefprozil and mixed anhydride of N-substituted-α-amino-p-hydroxy phenyl acetic acid salt (Dane salt). Detailed process was mentioned in experimental of the thesis.

Cefepime hydrochloride:

Fourth of the thesis includes the works for development of cost effective process of another cephalosporin antibiotic molecule Cefepime hydrochloride

Cefepime is a commercially valuable and therapeutically useful injectable forth generation Cephalosporin antibiotic effective in controlling diseases caused by a wide variety of Gram positive and Gram-negative microorganisms.

Thus a series of α-7 alkoxyimino derivatives having quaternized ammonium group in the 3- side chain were prepared. Among them, 7-[2-(2-aminotiazol-4yl)-2-(z)-methoxy-iminoacetamido]-3-[(1-methyl-1-pyrolidino)-methyl] ceph-3-em-4carboxylate (Cefepime) was found to be most promising in view of its antimicobial spectrum and other biological properties.

Selection of the synthetic scheme was made in parts from the available literature.. The methods having long and cumbersome synthetic steps was not opted for development. Based on the commercial availability of the raw materials, 7-ACA was selected as the key starting material.

Similarly the proposed scheme (for non infringing and industrially feasible process is selected based on Ludescher J., Wolf S. discloser and by taking 4-bromo- 2-methoxyimino-3-oxo-butyryl chloride instead of its chloro derivative and reaction in aqueous media by avoiding silylating agent which required the anhydrous condition which is difficult to achieve industrially.

Preparation of 7-amino-3-[(1-methyl-1-pyrrolidino)-methyl]ceph-3-em-4-carboxylate (3) (7-APC)

Here we provided the method for the preparation of 7-APC by silylating the 7-Amino Cephalosporanic with hexamethyl disilazane in presence of catalytic iodo trimethyl silane. The disilylated 7-ACA is reacted with iodo trimethyl silane and N-methyl pyrrolidine complex, which on acidification with hydrochloric acid gives crude 7-APC. The crude product is purified in acetone water to get pure 7-APC. The details of the process development and optimization of process parameters are described in the thesis

In order to get the better purity and description of the 7-APC was purified in acetone water mixture

Preparation of Cefepime hydrochloride

There are two method for preparation of [1-[[(6R,7R)-7-[[(2Z)-(4-bromo-2-methoxyimino-3-oxo-butyryl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methyl-pyrrolidinium hydrochloride
(**18,** BMPC) from 7-APC like silyl route and aqueous route as described Ludescher J., Wolf S. Silyl route requires the anhydrous reaction condition due to use of water sensitive silylating agent which are increases the manufacture cost hence aqueous route is preferred.

In the present work we provided the cheaper method in which 4-Bromo-2-methoxyimino-3-oxo-butyryl chloride was prepared from ammonium salt of 4-bromo-2-methoxyimino-3-oxo-butyric acid by phosphorous pentachloride in

dichloromethane and after completion of reaction dichloromethane was distilled out under reduced pressure the residual acid chloride solution was further used for condensation reaction in acetone-water at pH 5.4-5.8. The reaction solution was the acidified to pH 0.5-0.7 by concentrated hydrochloric acid to furnished the intermediate BMPC (**18**)

In order to get the better purity of the condensed intermediate (**18**,BMPC), its purification by acid base neutralization was carried out in acetone-water.

BMPC (**18**) is neutralized with base and the resulting free base is treated with thiourea, which on acidification gives Cefepime hydrochloride

To achieve the minimum impurity level purification of Cefepime hydrochloride was done by dissolving it in DMW and precipitating by addition of acetone. To meet the pharmacopial requirement it was again purified by dissolving in methanol and precipitating with acetone.

Preparation of Cefepime (6) by using (Z)-(2-aminothiazol-4-yl) methoxy-iminoacetic acid-2-mercaptobenzothiazolylester

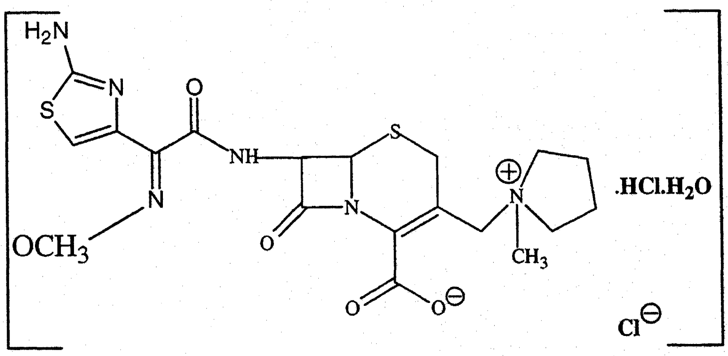
The present work also provides the another cost effective process for the preparation of Cefepime hydrochloride in which 7-Amino-3-[(1-methyl-1-pyrrolidino)-methyl]ceph-3-em-4-carboxylate (7-APC) was treated with (Z)-(2-aminothiazol-4-yl)methoxy-iminoacetic acid-2-mercaptobenzothiazolylester(**14**)

(MAEM) in presence of Triethyl amine base in Dimethyl formamide: Water Final product was isolated as hydrochloride salt in acetone: water by using hydrochloric acid.

Synthesis, isolation and characterization of impurities:

US Pharmacopoeia specifies that the purity of Cefepime should be between 90 to 105 %. However, most of the reported methods are associated with the formation of varying amounts of impurities and hence there is need to identify the cause of formation and characterization of impurities

Mainly two process impurities were observed in the Cefepime during its synthesis in the range of 0.2 to 1.5%, the molecular weight of these impurities were checked by LC-MS and from the molecular weight the structure of the impunities were illustrated as shown below structure **E-isomer of Cefepime** and **Lactone impurity of Cefepime** Efforts were made for the synthesis of these impurities.



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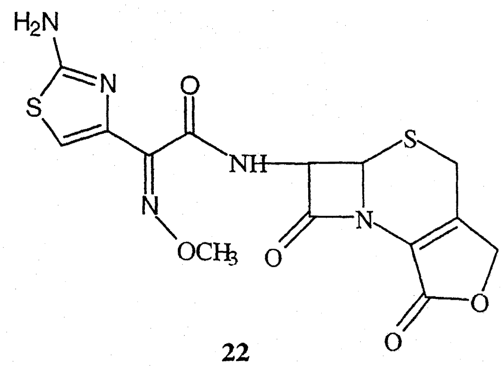

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The impurity E-isomer that is formed during the synthesis of Cefepime hydrochloride in the range of 0.5 to 1.5%.

Synthesis of the anti isomer of Cefepime (19) involved two steps in which acid chloride of 2-(Amino-4-thiazolyl)-2-(E) methoxy imino acetic acid was reacted with 7-amino-3- [(1-methyl-1-pyrrolidino)-methyl] ceph-3-em-4-carboxylate in aqueous acetone at pH 5.0 to 7.0. The resulting solution was acidified to pH 1.0 to 1.2 by hydrochloric acid and was precipitated by adding acetone to afforded anti isomer of Cefepime.It was characterized by NMR and confirmed by HPLC The detailed procedure is given in the thesis.

The impurity lactone is the process impurity formed during the synthesis of Cefepime hydrochloride. This impurity generally formed in very strong acidic or basic condition (Jeffery et al 1961) It was synthesized by acidic treatment of Cefepime hydrocloride in 0.1 N hydrochloric acid at room temperature for 5-6 h. Reaction mixture was then extracted in dichloromethane. The organic layer was concentrated under reduced pressure and the slowly added in di-isopropyl ether to

give the impure product containing impurity lactone. The impure compound was then purified by dissolving in dichloromethane and crystallization with di-isopropyl ether to afforded the impurity lactone in pure form which was confirmed by NMR.

Based on the work carried out for development of the cost effective process of the molecules Cefixime trihydrate, Cefprozil monohydrate and Cefepime hydrochloride was implemented successfully at industrial scale. The products obtained at same scale was compatible with marketed sample.

List of patent publications on Cephalosporins :

1) An improved process for the manufacture of 3-hydrxy Cephem derivative .

   Indian patent,(1999), **IN 182160.**

2) An improved process for the preparation of Penicillin antibiotics

   Indian patent,(2000), **IN 185067.**

3) An improved process for the preparation of Cephalosporin antibiotics

   Indian patent,(2000), **IN 184842.**

4) An improved process for the manufacture of 4-Bromo-2-Oxyimino Butyric acid and its derivatives **.(2003),WO03/045899 A1; (2005) US6846952**.

5) Process for preparation of 7-[α-amino-α-(4-hydroxyphenyl) acetamido]-3-(1-propen-1-yl)-3-cephem-4-carboxylic acid.

   (2004), **US Application No.10/801443.**

6) Temperature stable injectable Cephalosporin composition and process for preparation their of provisional application number **1149/MUM/2003.**

7) An improved process for the preparation of 7-amino-3-alkenyl-3-cephem-4-carboxylicacid compound, provisional application number **961/MUM/2004.**

8) A novel intermediate for preparation of Cefepime, provisional application number **1382/MUM/2004.**

9) An improved process for the preparation of Cefexime, provisional application number **1384/MUM/2004.**